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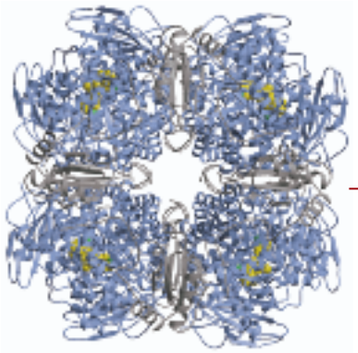

chapter 20

CARBOHYDRATE BIOSYNTHESIS IN PLANTS AND BACTERIA

20.1 Photosynthetic Carbohydrate Synthesis *751*

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. . . the discovery of the long-lived isotope of carbon, carbon-14, by Samuel Ruben and Martin Kamen in 1940 provided the ideal tool for the tracing of the route along which carbon dioxide travels on its way to carbohydrate.

—Melvin Calvin, Nobel Address, 1961

We have now reached a turning point in our study of cellular metabolism. Thus far in Part II we have described how the major metabolic fuels—carbohydrates, fatty acids, and amino acids—are degraded through converging *catabolic* pathways to enter the citric acid cycle and yield their electrons to the respiratory chain, and how this exergonic flow of electrons to oxygen is coupled to the endergonic synthesis of ATP. We now turn to *anabolic* pathways, which use chemical energy in the form of ATP and NADH or NADPH to synthesize cellular components from simple precursor molecules. Anabolic pathways are generally reductive rather than oxidative. Catabolism and anabolism proceed simultaneously in a dynamic steady state, so the energy-yielding degradation of cellular components is counterbalanced by biosynthetic processes, which create and maintain the intricate orderliness of living cells.

Plants must be especially versatile in their handling of carbohydrates, for several reasons. First, plants are autotrophs, able to convert inorganic carbon (as CO_2) into organic compounds. Second, biosynthesis occurs primarily in plastids, membrane-bounded organelles unique to plants, and the movement of intermediates between cellular compartments is an important aspect of metabolism. Third, plants are not motile: they cannot move to find better supplies of water, sunlight, or nutrients. They must have sufficient metabolic flexibility to allow them to adapt to changing conditions in the place where they are rooted. Finally, plants have thick cell walls made of carbohydrate polymers, which must be assembled outside the plasma membrane and which constitute a significant proportion of the cell's carbohydrate.

The chapter begins with a description of the process by which CO_2 is assimilated into trioses and hexoses, then considers photorespiration, an important side reaction during CO_2 fixation, and the ways in which certain plants avoid this side reaction. We then look at how the biosynthesis of sucrose (for sugar transport) and starch (for energy storage) is accomplished by mechanisms analogous to those employed by animal cells to make glycogen. The next topic is the synthesis of the cellulose of plant cell walls and the peptidoglycan of bacterial cell walls, illustrating the problems of energy-dependent biosynthesis outside the plasma membrane. Finally, we discuss how the various pathways that share pools of common intermediates are segregated within organelles yet integrated with one another.

20.1 Photosynthetic Carbohydrate Synthesis

The synthesis of carbohydrates in animal cells always employs precursors having at least three carbons, all of which are less oxidized than the carbon in CO_2. Plants

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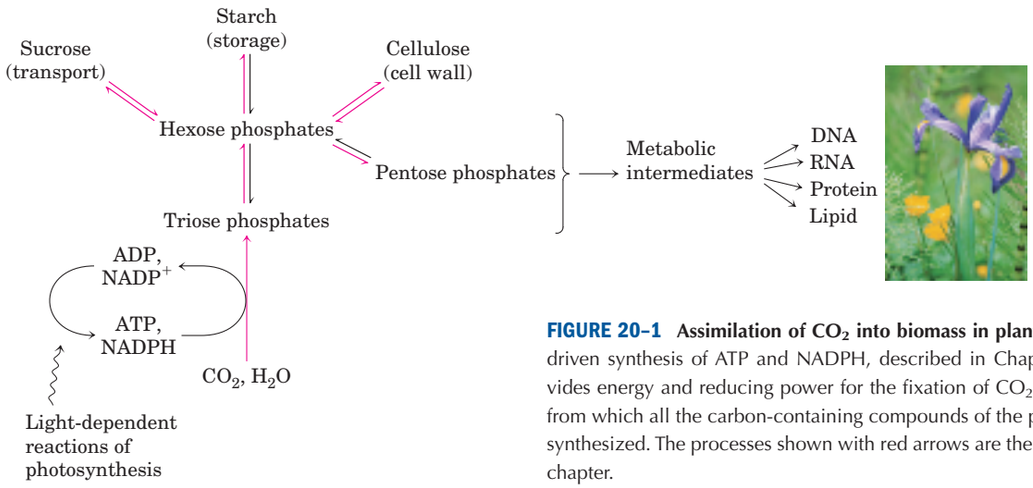

FIGURE 20–1 Assimilation of CO_2 into biomass in plants. The light-driven synthesis of ATP and NADPH, described in Chapter 19, provides energy and reducing power for the fixation of CO_2 into trioses, from which all the carbon-containing compounds of the plant cell are synthesized. The processes shown with red arrows are the focus of this chapter.

and photosynthetic microorganisms, by contrast, can synthesize carbohydrates from CO_2 and water, reducing CO_2 at the expense of the energy and reducing power furnished by the ATP and NADPH that are generated by the light-dependent reactions of photosynthesis (Fig. 20–1). Plants (and other autotrophs) can use CO_2 as the sole source of the carbon atoms required for the biosynthesis of cellulose and starch, lipids and proteins, and the many other organic components of plant cells. By contrast, heterotrophs cannot bring about the net reduction of CO_2 to achieve a net synthesis of glucose.

Green plants contain in their chloroplasts unique enzymatic machinery that catalyzes the conversion of CO_2 to simple (reduced) organic compounds, a process called **CO_2 assimilation.** This process has also been called **CO_2 fixation** or **carbon fixation,** but we reserve these terms for the specific reaction in which CO_2 is incorporated (fixed) into a three-carbon organic compound, the triose phosphate 3-phosphoglycerate. This simple product of photosynthesis is the precursor of more complex biomolecules, including sugars, polysaccharides, and the metabolites derived from them, all of which are synthesized by metabolic pathways similar to those of animal tissues. Carbon dioxide is assimilated via a cyclic pathway, its key intermediates constantly regenerated. The pathway was elucidated in the early 1950s by Melvin Calvin, Andrew Benson, and James A. Bassham, and is often called the **Calvin cycle** or, more descriptively, the **photosynthetic carbon reduction cycle.**

Melvin Calvin,
1911–1997

Carbohydrate metabolism is more complex in plant cells than in animal cells or in nonphotosynthetic microorganisms. In addition to the universal pathways of glycolysis and gluconeogenesis, plants have the unique reaction sequences for reduction of CO_2 to triose phosphates and the associated reductive pentose phosphate pathway—all of which must be coordinately regulated to ensure proper allocation of carbon to energy production and synthesis of starch and sucrose. Key enzymes are regulated, as we shall see, by (1) reduction of disulfide bonds by electrons flowing from photosystem I and (2) changes in pH and Mg^{2+} concentration that result from illumination. When we look at other aspects of plant carbohydrate metabolism, we also find enzymes that are modulated by (3) conventional allosteric regulation by one or more metabolic intermediates and (4) covalent modification (phosphorylation).

Plastids Are Organelles Unique to Plant Cells and Algae

Most of the biosynthetic activities in plants (including CO_2 assimilation) occur in **plastids,** a family of self-reproducing organelles bounded by a double membrane and containing a small genome that encodes some of their proteins. Most proteins destined for plastids are encoded in nuclear genes, which are transcribed and translated like other nuclear genes; then the proteins are imported into plastids. Plastids reproduce by binary fission, replicating their genome (a single circular DNA molecule) and using their own enzymes and ribosomes to synthesize the proteins encoded by that genome. **Chloroplasts** (see Fig. 19–38) are the sites of CO_2 assimilation. The enzymes for this process are contained in the stroma, the soluble phase bounded by the inner chloroplast membrane. **Amyloplasts** are colorless plastids (that is, they lack chlorophyll and other pig-

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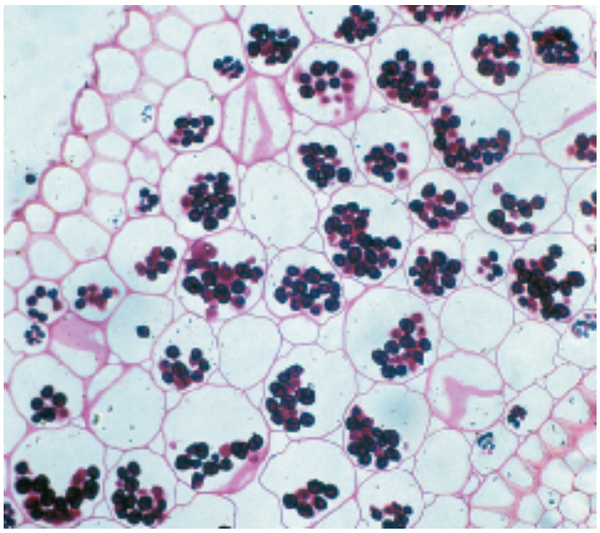

FIGURE 20–2 Amyloplasts filled with starch (dark granules) are stained with iodine in this section of *Ranunculus* root cells. Starch granules in various tissues range from 1 to 100 μm in diameter.

ments found in chloroplasts). They have no internal membranes analogous to the photosynthetic membranes (thylakoids) of chloroplasts, and in plant tissues rich in starch these plastids are packed with starch granules (Fig. 20–2). Chloroplasts can be converted to **proplastids** by the loss of their internal membranes and chlorophyll, and proplastids are interconvertible with amyloplasts (Fig. 20–3). In turn, both amyloplasts and proplastids can develop into chloroplasts. The relative proportions of the plastid types depend on the type of plant tissue and on the intensity of illumination. Cells of green leaves are rich in chloroplasts, whereas amyloplasts dominate in nonphotosynthetic tissues that store starch in large quantities, such as potato tubers.

The inner membranes of all types of plastids are impermeable to polar and charged molecules. Traffic across these membranes is mediated by sets of specific transporters.

Carbon Dioxide Assimilation Occurs in Three Stages

The first stage in the assimilation of CO_2 into biomolecules (Fig. 20–4) is the **carbon-fixation reaction:** condensation of CO_2 with a five-carbon acceptor, **ribulose 1,5-bisphosphate,** to form two molecules of **3-phosphoglycerate.** In the second stage, the 3-phosphoglycerate is reduced to triose phosphates. Overall, three molecules of CO_2 are fixed to three molecules of ribulose 1,5-bisphosphate to form six molecules of glyceraldehyde 3-phosphate (18 carbons) in equilibrium with dihydroxyacetone phosphate. In the third stage, five of the six molecules of triose phosphate (15 carbons) are used to regenerate three molecules of ribulose 1,5-bisphosphate (15 carbons), the starting material. The sixth molecule of triose phosphate, the net product of photosynthesis, can be used to make hexoses for fuel and building materials, sucrose for transport to nonphotosynthetic tissues, or starch for storage. Thus the overall process is cyclical, with the continuous conversion of CO_2 to triose and hexose phosphates. Fructose 6-phosphate is a key intermediate in stage 3 of CO_2 assimilation; it stands at a branch point, leading either to regeneration of ribulose 1,5-bisphosphate or to synthesis of starch. The pathway from hexose phosphate to pentose bisphosphate involves many of the same reactions used in animal cells for the conversion of pentose phosphates to hexose phosphates during the nonoxidative phase of the **pentose phosphate pathway** (see Fig. 14–22). In the photosynthetic assimilation of CO_2, essentially the same set of reactions operates in the other direction, converting hexose phosphates to pentose phosphates. This **reductive pentose phosphate cycle** uses the same enzymes as the oxidative pathway, and several more enzymes that make the reductive cycle irreversible. All 13 enzymes of the pathway are in the chloroplast stroma.

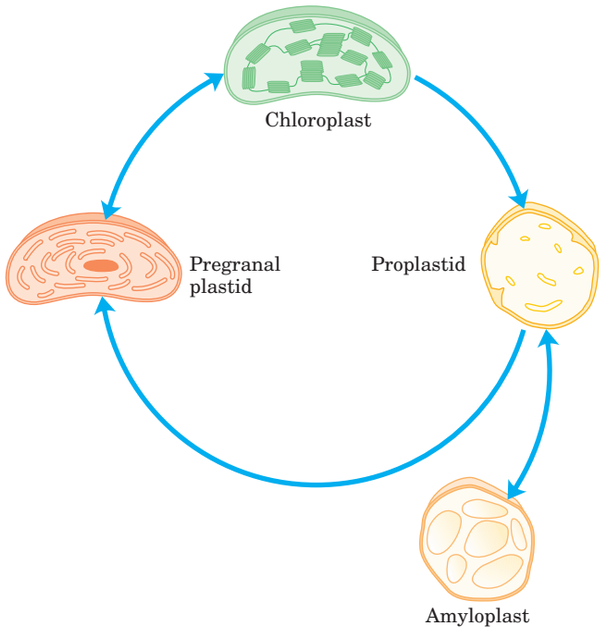

FIGURE 20–3 Plastids: their origins and interconversions. All types of plastids are bounded by a double membrane, and some (notably the mature chloroplast) have extensive internal membranes. The internal membranes can be lost (when a mature chloroplast becomes a proplastid) and resynthesized (as a proplastid gives rise to a pregranal plastid and then a mature chloroplast). Proplastids in nonphotosynthetic tissues (such as root) give rise to amyloplasts, which contain large quantities of starch. All plant cells have plastids, and these organelles are the site of other important processes, including the synthesis of essential amino acids, thiamine, pyridoxal phosphate, flavins, and vitamins A, C, E, and K.

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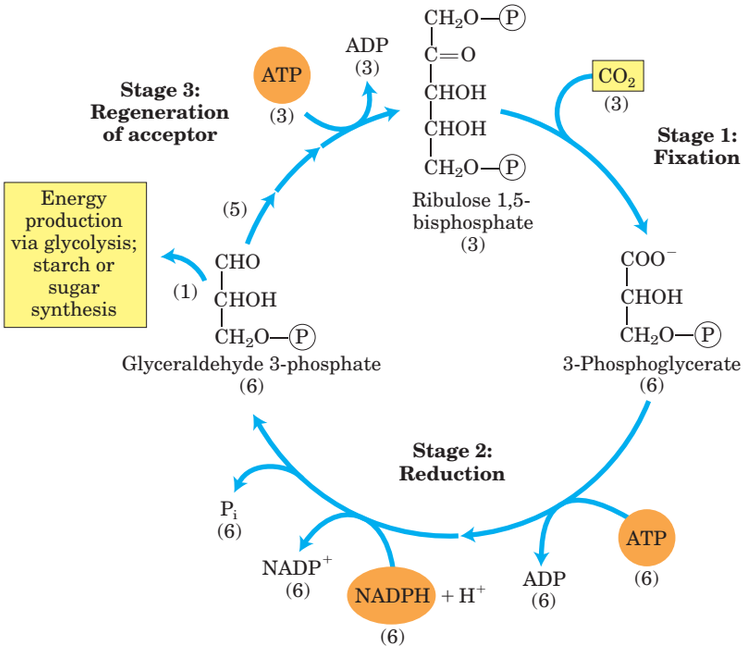

FIGURE 20–4 The three stages of CO_2 assimilation in photosynthetic organisms. Stoichiometries of three key intermediates (numbers in parentheses) reveal the fate of carbon atoms entering and leaving the cycle. As shown here, three CO_2 are fixed for the net synthesis of one molecule of glyceraldehyde 3-phosphate. This cycle is the photosynthetic carbon reduction cycle, or the Calvin cycle.

Stage 1: Fixation of CO_2 into 3-Phosphoglycerate An important clue to the nature of the CO_2-assimilation mechanisms in photosynthetic organisms came in the late 1940s. Calvin and his associates illuminated a suspension of green algae in the presence of radioactive carbon dioxide ($^{14}CO_2$) for just a few seconds, then quickly killed the cells, extracted their contents, and with the help of chromatographic methods searched for the metabolites in which the labeled carbon first appeared. The first compound that became labeled was **3-phosphoglycerate,** with the ^{14}C predominantly located in the carboxyl carbon atom. These experiments strongly suggested that 3-phosphoglycerate is an early intermediate in photosynthesis. The many plants in which this three-carbon compound is the first intermediate are called **C_3 plants,** in contrast with the C_4 plants described below.

The enzyme that catalyzes incorporation of CO_2 into an organic form is **ribulose 1,5-bisphosphate carboxylase/oxygenase,** a name mercifully shortened to **rubisco.** As a carboxylase, rubisco catalyzes the covalent attachment of CO_2 to the five-carbon sugar ribulose 1,5-bisphosphate and cleavage of the unstable six-carbon intermediate to form two molecules of 3-phosphoglycerate, one of which bears the carbon introduced as CO_2 in its carboxyl group (Fig. 20–4). The enzyme's oxygenase activity is discussed in Section 20.2.

Plant rubisco, the crucial enzyme in the production of biomass from CO_2, has a complex structure (Fig. 20–5a), with eight identical large subunits (M_r 53,000; encoded in the chloroplast genome, or plastome), each containing a catalytic site, and eight identical small subunits (M_r 14,000; encoded in the nuclear genome) of uncertain function. The rubisco of photosynthetic bacteria is simpler in structure, having two subunits that in many respects resemble the large subunits of the plant enzyme (Fig. 20–5b). This similarity is consistent with the endosymbiont hypothesis for the origin of chloroplasts (p. 35). The plant enzyme has an exceptionally low turnover number; only three molecules of CO_2 are fixed per second per molecule of rubisco at 25 °C. To achieve high rates of CO_2 fixation, plants therefore need large amounts of this enzyme. In fact, rubisco makes up almost 50% of soluble protein in chloroplasts and is probably one of the most abundant enzymes in the biosphere.

Central to the proposed mechanism for plant rubisco is a carbamoylated Lys side chain with a bound Mg^{2+} ion. The Mg^{2+} ion brings together and orients the reactants at the active site (Fig. 20–6) and polarizes the CO_2, opening it to nucleophilic attack by the five-carbon enediolate reaction intermediate formed on the enzyme (Fig. 20–7). The resulting six-carbon intermediate breaks down to yield two molecules of 3-phosphoglycerate.

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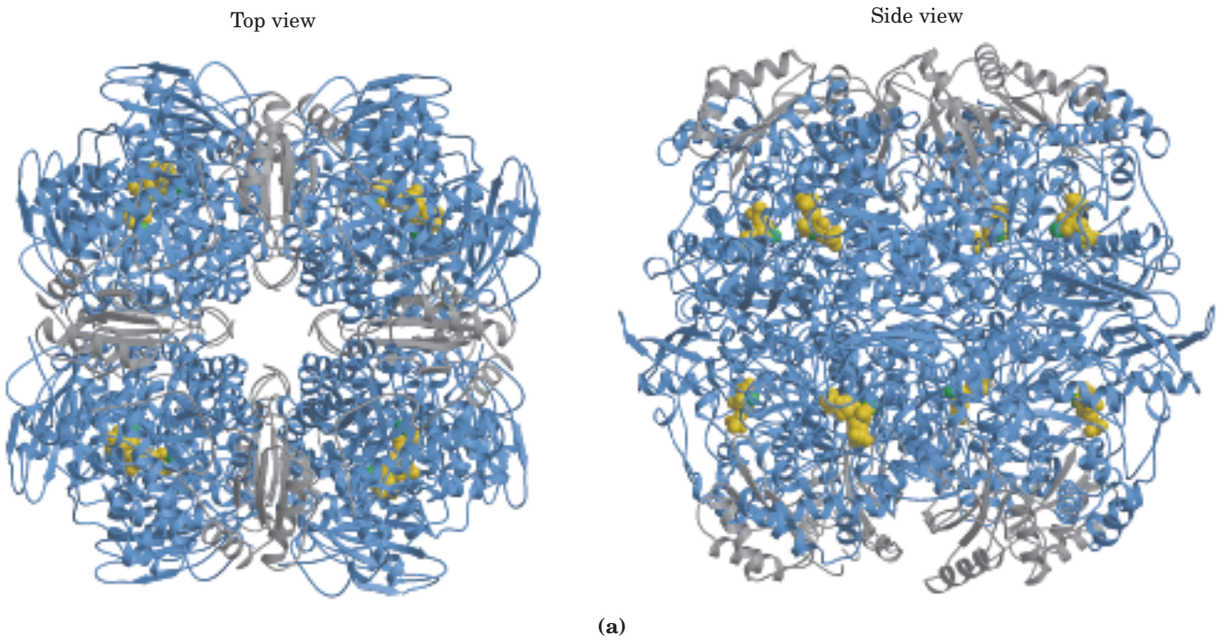

FIGURE 20–5 Structure of ribulose 1,5-bisphosphate carboxylase (rubisco). (a) Top and side view of a ribbon model of rubisco from spinach (PDB ID 8RUC). The enzyme has eight large subunits (blue) and eight small ones (gray), tightly packed into a structure of M_r 550,000. Rubisco is present at a concentration of about 250 mg/mL in the chloroplast stroma, corresponding to an extraordinarily high concentration of active sites (~4 mM). Amino acid residues of the active site are shown in yellow, Mg^{2+} in green. **(b)** Ribbon model of rubisco from the bacterium *Rhodospirillum rubrum* (PDB ID 9RUB). The subunits are in gray and blue. A Lys residue at the active site that is carboxylated to a carbamate in the active enzyme is shown in red. The substrate, ribulose 1,5-bisphosphate, is yellow; Mg^{2+} is green.

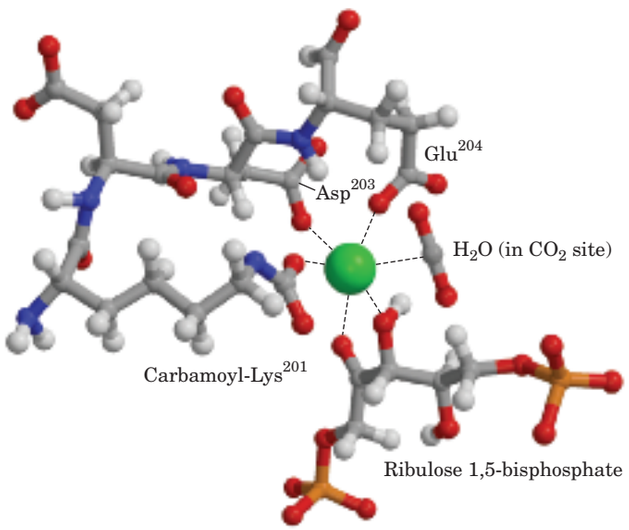

FIGURE 20–6 Central role of Mg^{2+} in the catalytic mechanism of rubisco. (Derived from PDB ID 1RXO) Mg^{2+} is coordinated in a roughly octahedral complex with six oxygen atoms: one oxygen in the carbamate on Lys^{201}; two in the carboxyl groups of Glu^{204} and Asp^{203}; two at C-2 and C-3 of the substrate, ribulose 1,5-bisphosphate; and one in the other substrate, CO_2. A water molecule occupies the Co2–binding site in this crystal structure. (Residue numbers refer to the spinach enzyme.)

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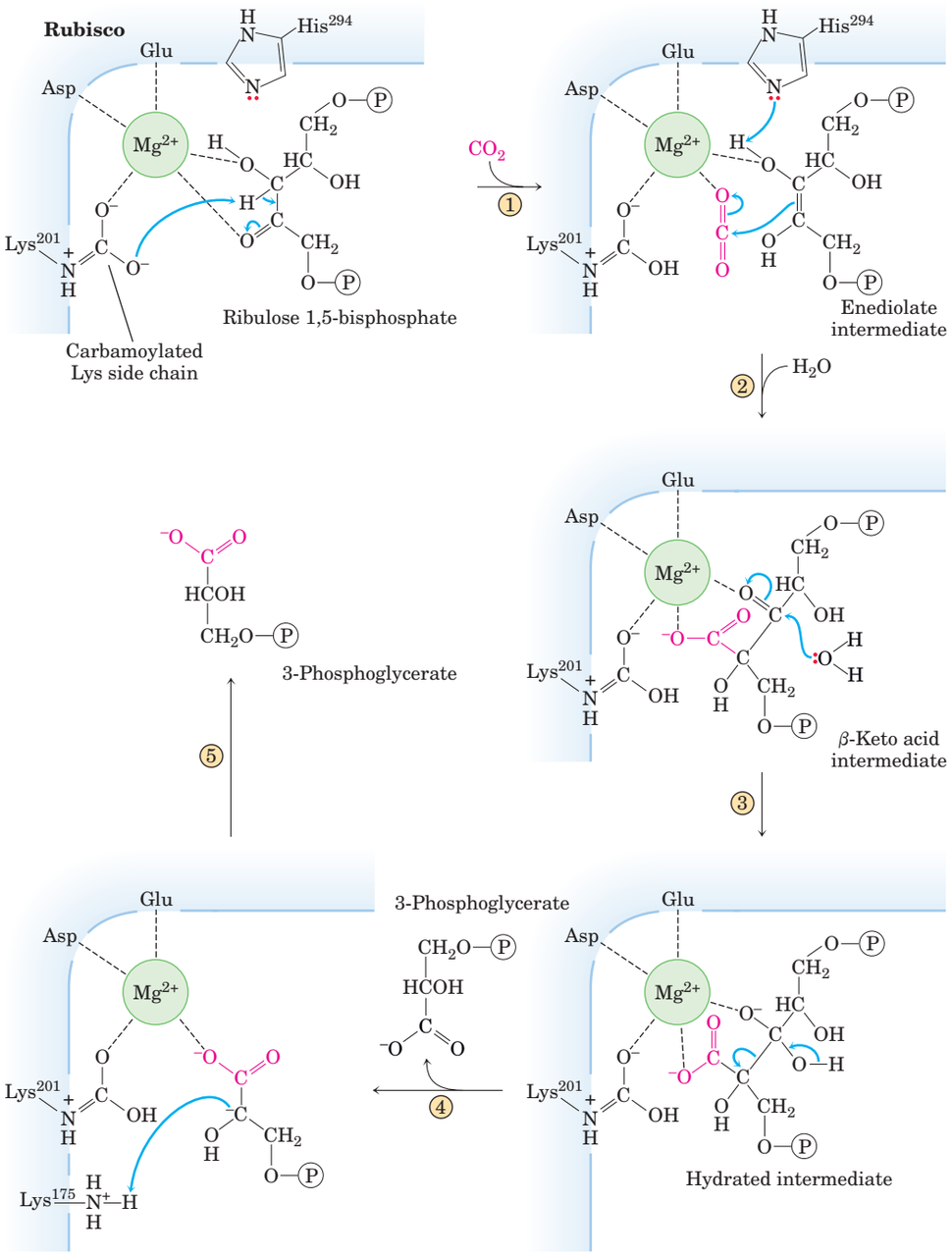

MECHANISM FIGURE 20–7 First stage of CO_2 assimilation: rubisco's carboxylase activity. The CO_2-fixation reaction is catalyzed by ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco). (1) Ribulose 1,5-bisphosphate forms an enediolate at the active site. (2) CO_2, polarized by the proximity of the Mg^{2+} ion, undergoes nucleophilic attack by the enediolate, producing a branched six-carbon sugar. (3) Hydroxylation at C-3 of this sugar, followed by aldol cleavage (4), forms one molecule of 3-phosphoglycerate, which leaves the enzyme active site. (5) The carbanion of the remaining three-carbon fragment is protonated by the nearby side chain of Lys^{175}, generating a second molecule of 3-phosphoglycerate. The overall reaction therefore accomplishes the combination of one CO_2 and one ribulose 1,5-bisphosphate to form two molecules of 3-phosphoglycerate, one of which contains the carbon atom from CO_2 (red). **Rubisco Mechanism; Rubisco Tutorial**

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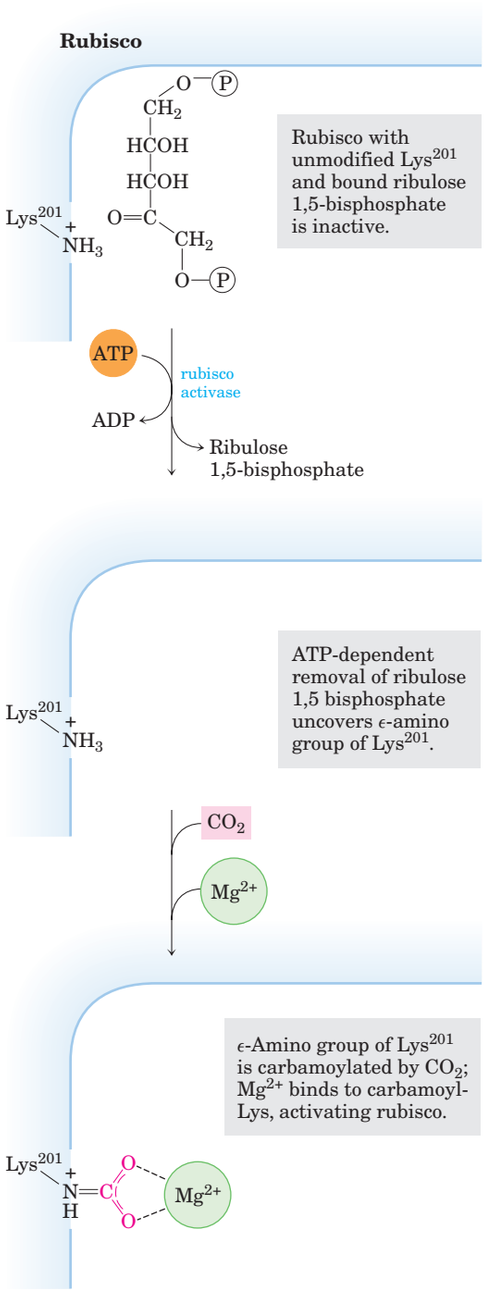
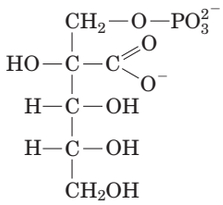


FIGURE 20–8 Role of rubisco activase in the carbamoylation of Lys^{201} of rubisco. When the substrate ribulose 1,5-bisphosphate is bound to the active site, Lys^{201} is not accessible. Rubisco activase couples ATP hydrolysis to expulsion of the bound sugar bisphosphate, exposing Lys^{201}; this Lys residue can now be carbamoylated with CO_2 in a reaction that is apparently not enzyme-mediated. Mg^{2+} is attracted to and binds to the negatively charged carbamoyl-Lys, and the enzyme is thus activated.

As the catalyst for the first step of photosynthetic CO_2 assimilation, rubisco is a prime target for regulation. The enzyme is inactive until carbamoylated on the ε amino group of Lys^{201} (Fig. 20–8). Ribulose 1,5-bisphosphate inhibits carbamoylation by binding tightly to the active site and locking the enzyme in the "closed" conformation, in which Lys^{201} is inaccessible. **Rubisco activase** overcomes the inhibition by promoting ATP-dependent release of the ribulose 1,5-bisphosphate, exposing the Lys amino group to nonenzymatic carbamoylation by CO_2; this is followed by Mg^{2+} binding, which activates the rubisco. Rubisco activase in some species is activated by light through a redox mechanism (see Fig. 20–19).

Another regulatory mechanism involves the "nocturnal inhibitor" 2-carboxyarabinitol 1-phosphate, a naturally occurring transition-state analog (see Box 6–3) with a structure similar to that of the β-keto acid intermediate of the rubisco reaction (Fig. 20–7; see also Fig. 20–20). This compound, synthesized in the dark in some plants, is a potent inhibitor of carbamoylated rubisco. It is either broken down when light returns or is expelled by rubisco activase, activating the rubisco.



2-Carboxyarabinitol 1-phosphate

Stage 2: Conversion of 3-Phosphoglycerate to Glyceraldehyde 3-Phosphate The 3-phosphoglycerate formed in stage 1 is converted to glyceraldehyde 3-phosphate in two steps that are essentially the reversal of the corresponding steps in glycolysis, with one exception: the nucleotide cofactor for the reduction of 1,3-bisphosphoglycerate is NADPH rather than NADH (Fig. 20–9). The chloroplast stroma contains all the glycolytic enzymes except phosphoglycerate mutase. The stromal and cytosolic enzymes are isozymes; both sets of enzymes catalyze the same reactions, but they are the products of different genes.

In the first step of stage 2, the stromal **3-phosphoglycerate kinase** catalyzes the transfer of a phosphoryl group from ATP to 3-phosphoglycerate, yielding 1,3-bisphosphoglycerate. Next, NADPH donates electrons in a reduction catalyzed by the chloroplast-specific isozyme of **glyceraldehyde 3-phosphate dehydrogenase,** producing glyceraldehyde 3-phosphate and P_i. Triose phosphate isomerase then interconverts glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.

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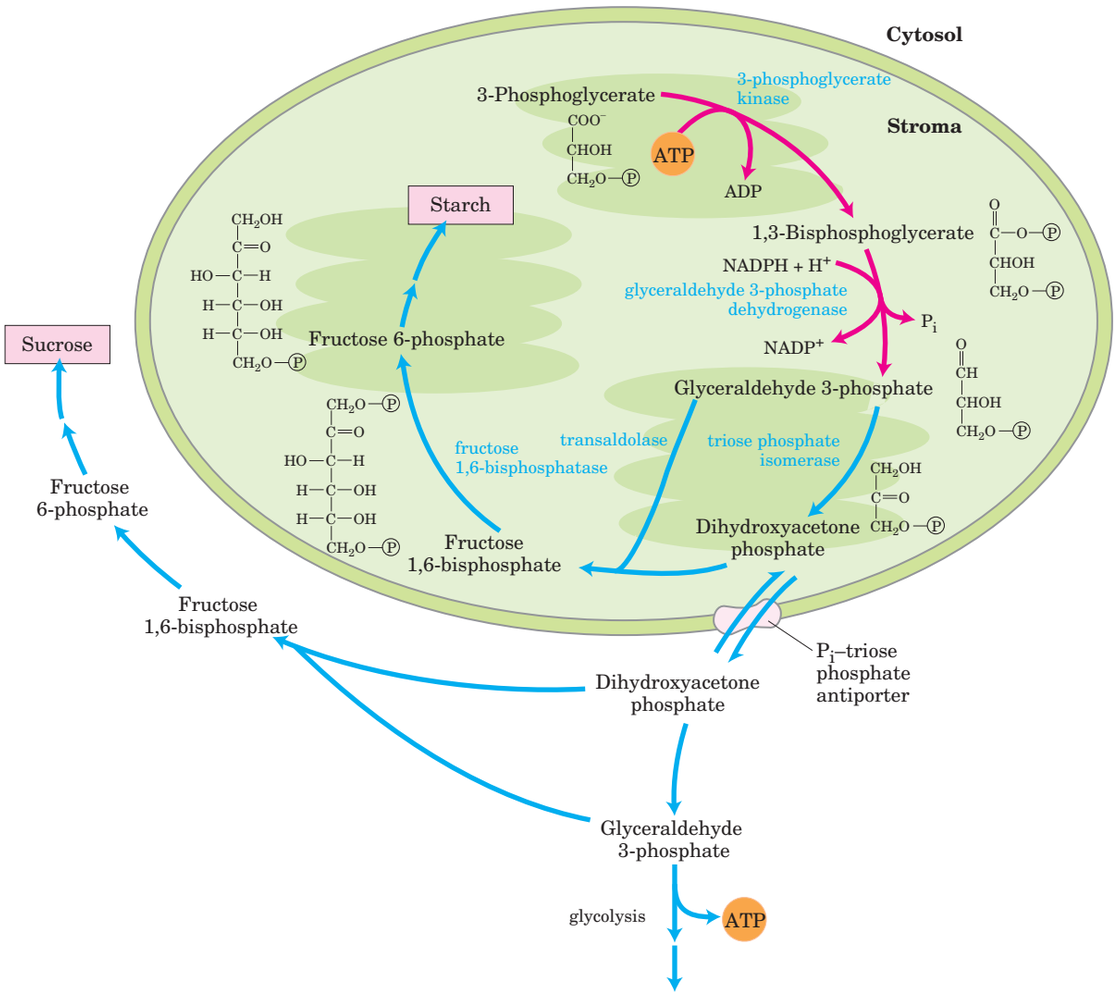

FIGURE 20–9 Second stage of CO_2 assimilation. 3-Phosphoglycerate is converted to glyceraldehyde 3-phosphate (red arrows). Also shown are the alternative fates of the fixed carbon of glyceraldehyde 3-phosphate (blue arrows). Most of the glyceraldehyde 3-phosphate is recycled to ribulose 1,5-bisphosphate as shown in Figure 20–10. A small fraction of the "extra" glyceraldehyde 3-phosphate may be used immediately as a source of energy, but most is converted to sucrose for transport or is stored in the chloroplast as starch. In the latter case, glyceraldehyde 3-phosphate condenses with dihydroxyacetone phosphate in the stroma to form fructose 1,6-bisphosphate, a precursor of starch. In other situations the glyceraldehyde 3-phosphate is converted to dihydroxyacetone phosphate, which leaves the chloroplast via a specific transporter (see Fig. 20–15) and, in the cytosol, can be degraded glycolytically to provide energy or used to form fructose 6-phosphate and hence sucrose.

Most of the triose phosphate thus produced is used to regenerate ribulose 1,5-bisphosphate; the rest is either converted to starch in the chloroplast and stored for later use or immediately exported to the cytosol and converted to sucrose for transport to growing regions of the plant. In developing leaves, a significant portion of the triose phosphate may be degraded by glycolysis to provide energy.

Stage 3: Regeneration of Ribulose 1,5-Bisphosphate from Triose Phosphates The first reaction in the assimilation of CO_2 into triose phosphates consumes ribulose 1,5-bisphosphate and, for continuous flow of CO_2 into carbohydrate, ribulose 1,5-bisphosphate must be constantly regenerated. This is accomplished in a series of reactions (Fig. 20–10) that, together with stages 1 and 2, constitute the cyclic pathway shown in Figure 20–4. The product of

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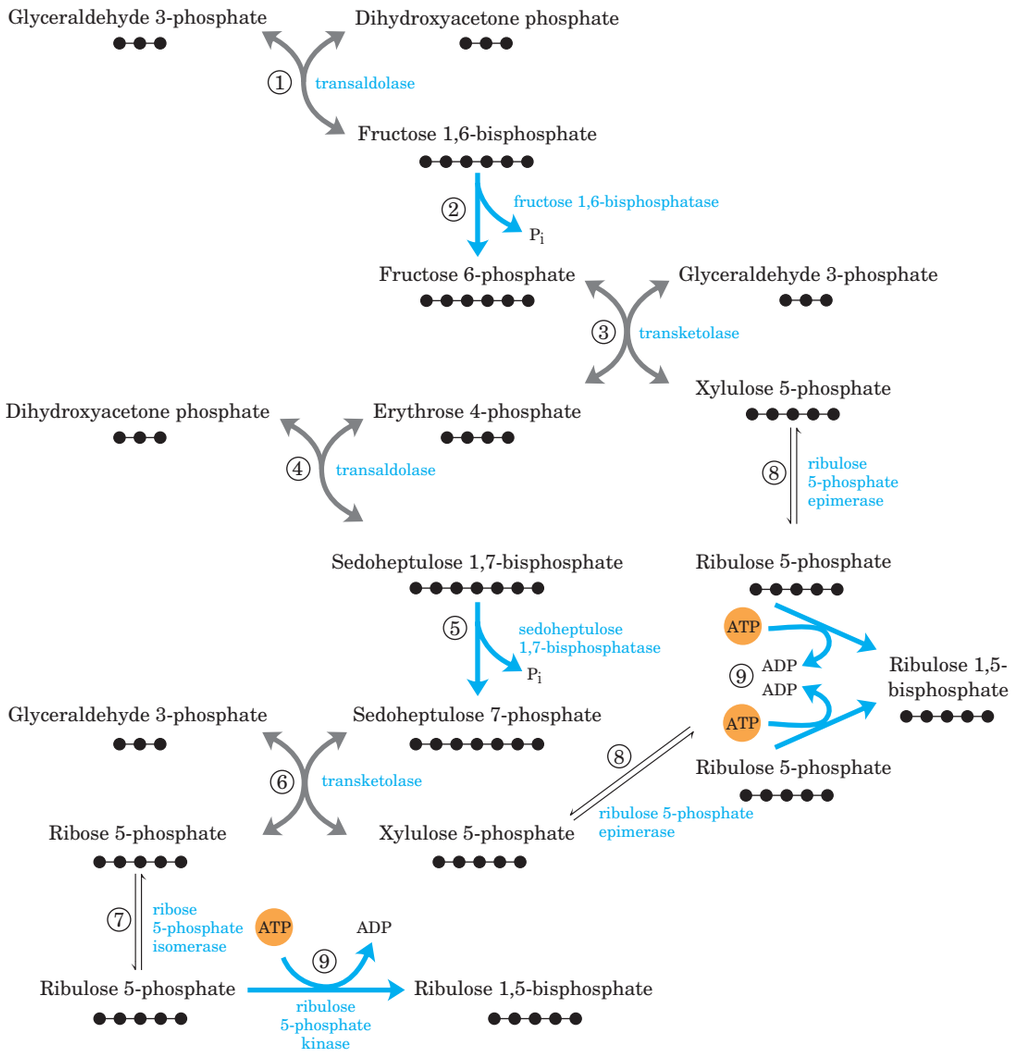

FIGURE 20–10 Third stage of CO_2 assimilation. This schematic diagram shows the interconversions of triose phosphates and pentose phosphates. Black dots represent the number of carbons in each compound. The starting materials are glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Reactions catalyzed by transaldolase (① and ④) and transketolase (③ and ⑥) produce pentose phosphates that are converted to ribulose 1,5-bisphosphate—ribose 5-phosphate by ribose 5-phosphate isomerase (⑦) and xylulose 5-phosphate by ribulose 5-phosphate epimerase (⑧). In step ⑨, ribulose 5-phosphate is phosphorylated, regenerating ribulose 1,5-bisphosphate. The steps with blue arrows are exergonic and make the whole process irreversible: steps ② fructose 1,6-bisphosphatase, ⑤ sedoheptulose bisphosphatase, and ⑨ ribulose 5-phosphate kinase.

the first assimilation reaction (3-phosphoglycerate) thus undergoes transformations that regenerate ribulose 1,5-bisphosphate. The intermediates in this pathway include three-, four-, five-, six-, and seven-carbon sugars. In the following discussion, all step numbers refer to Figure 20–10.

Steps ① and ④ are catalyzed by the same enzyme, **transaldolase.** It first catalyzes the reversible condensation of glyceraldehyde 3-phosphate with dihydroxyacetone phosphate, yielding fructose 1,6-bisphosphate (step ①); this is cleaved to fructose 6-phosphate and P_i by fructose 1,6-bisphosphatase (FBPase-1) in step ②. The reaction is strongly exergonic and essentially irreversible. Step ③ is catalyzed by **transketolase,** which contains thiamine pyrophosphate (TPP) as its prosthetic group (see Fig. 14–13a) and requires Mg^{2+}.

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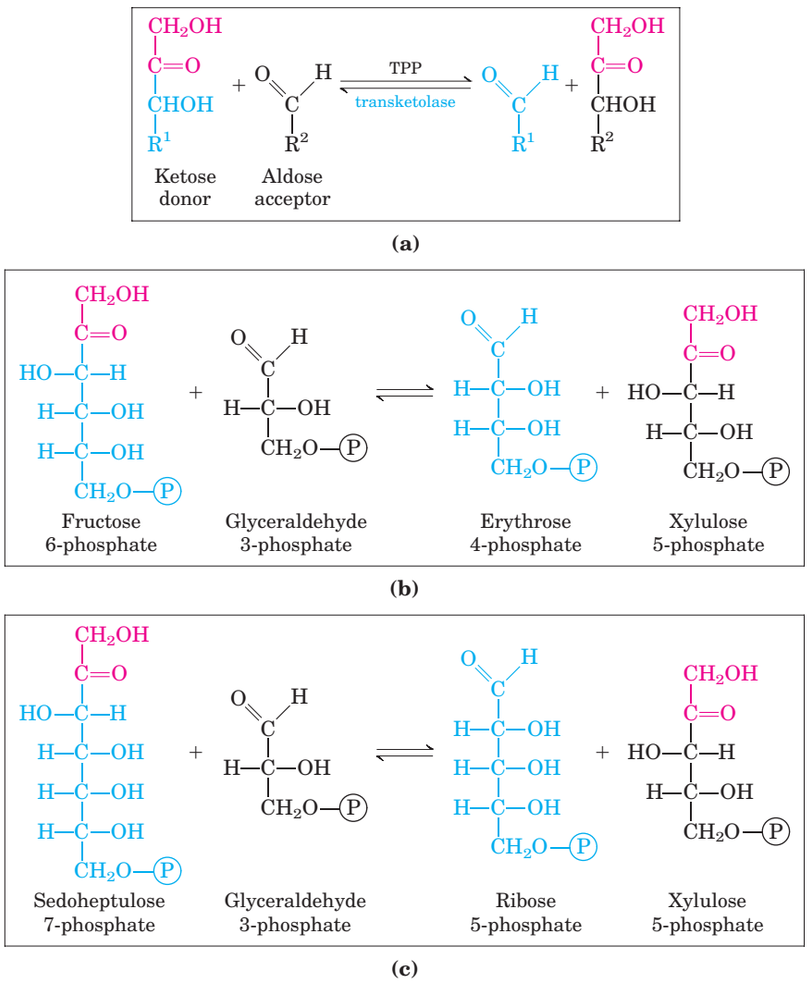

FIGURE 20–11 Transketolase-catalyzed reactions of the Calvin cycle. (a) General reaction catalyzed by transketolase: the transfer of a two-carbon group, carried temporarily on enzyme-bound TPP, from a ketose donor to an aldose acceptor. **(b)** Conversion of a hexose and a triose to a four-carbon and a five-carbon sugar (step ③ of Fig. 20–10). **(c)** Conversion of seven-carbon and three-carbon sugars to two pentoses (step ⑥ of Fig. 20–10).

Transketolase catalyzes the reversible transfer of a 2-carbon ketol group ($CH_2OH—CO—$) from a ketose phosphate donor, fructose 6-phosphate, to an aldose phosphate acceptor, glyceraldehyde 3-phosphate (Fig. 20–11a, b), forming the pentose xylulose 5-phosphate and the tetrose erythrose 4-phosphate. In step ④, transaldolase acts again, combining erythrose 4-phosphate with dihydroxyacetone phosphate to form the seven-carbon **sedoheptulose 1,7-bisphosphate.** An enzyme unique to plastids, sedoheptulose 1,7-bisphosphatase, converts the bisphosphate to sedoheptulose 7-phosphate (step ⑤); this is the second irreversible reaction in the pathway. Transketolase now acts again, converting sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate to two pentose phosphates in step ⑥ (Fig. 20–11c). Figure 20–12 shows how a two-carbon fragment is temporarily carried on the transketolase cofactor TPP and condensed with the three carbons of glyceraldehyde 3-phosphate in step ⑥.

The pentose phosphates formed in the transketolase reactions—ribose 5-phosphate and xylulose 5-phosphate—are converted to **ribulose 5-phosphate** (steps ⑦ and ⑧), which in the final step (⑨) of the cycle is phosphorylated to ribulose 1,5-bisphosphate by ribulose 5-phosphate kinase (Fig. 20–13). This is the third very exergonic reaction of the pathway, as the phosphate anhydride bond in ATP is swapped for a phosphate ester in ribulose 1,5-bisphosphate.

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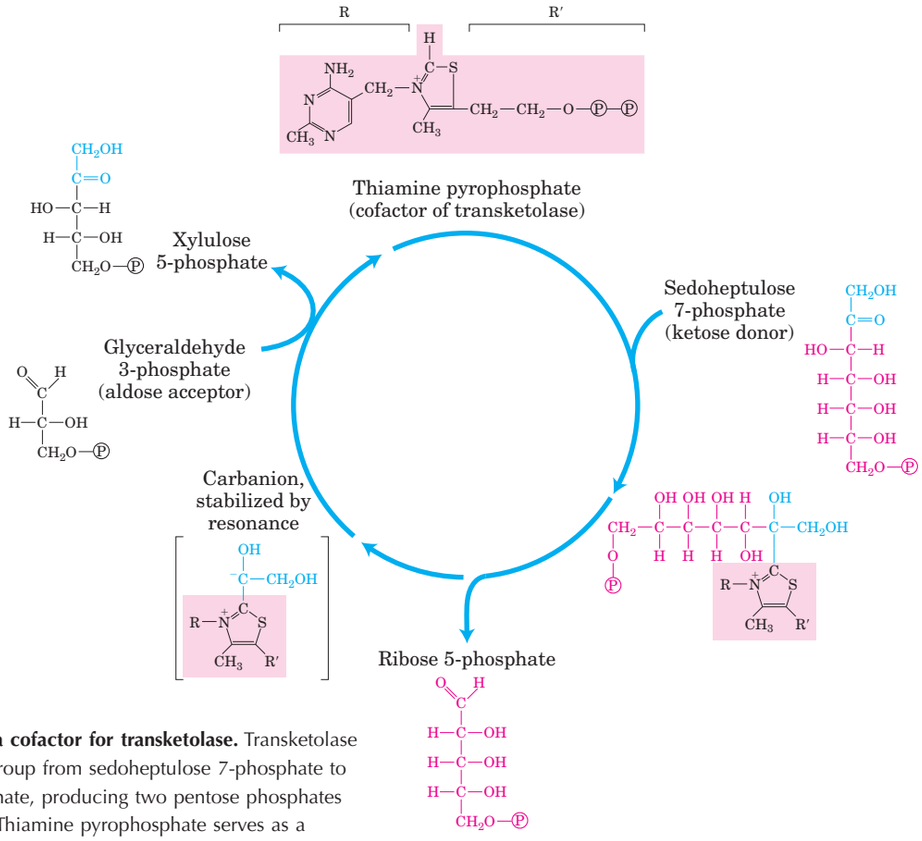

FIGURE 20–12 TPP as a cofactor for transketolase. Transketolase transfers a two-carbon group from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate, producing two pentose phosphates (step ⑥ in Fig. 20–10). Thiamine pyrophosphate serves as a temporary carrier of the two-carbon unit and as an electron sink (see Fig. 14–13) to facilitate the reactions.

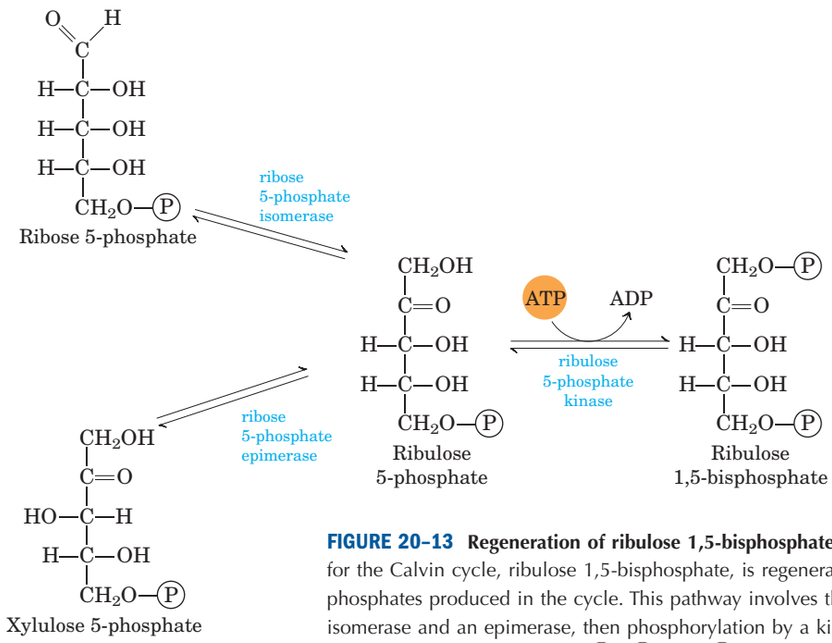

FIGURE 20–13 Regeneration of ribulose 1,5-bisphosphate. The starting material for the Calvin cycle, ribulose 1,5-bisphosphate, is regenerated from two pentose phosphates produced in the cycle. This pathway involves the action of an isomerase and an epimerase, then phosphorylation by a kinase, with ATP as phosphate group donor (steps ⑦, ⑧, and ⑨ of Fig. 20–10).

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Synthesis of Each Triose Phosphate from CO_2 Requires Six NADPH and Nine ATP

The net result of three turns of the Calvin cycle is the conversion of three molecules of CO_2 and one molecule of phosphate to a molecule of triose phosphate. The stoichiometry of the overall path from CO_2 to triose phosphate, with regeneration of ribulose 1,5-bisphosphate, is shown in Figure 20–14. Three molecules of ribulose 1,5-bisphosphate (a total of 15 carbons) condense with three CO_2 (3 carbons) to form six molecules of 3-phosphoglycerate (18 carbons). These six molecules of 3-phosphoglycerate are reduced to six molecules of glyceraldehyde 3-phosphate (which is in equilibrium with dihydroxyacetone phosphate), with the expenditure of six ATP (in the synthesis of 1,3-bisphosphoglycerate) and six NADPH (in the reduction of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate). The isozyme of glyceraldehyde 3-phosphate dehydrogenase present in chloroplasts can use NADP as its electron carrier and normally functions in the direction of 1,3-bisphosphoglycerate reduction. The cytosolic isozyme uses NAD, as does the glycolytic enzyme of animals and other eukaryotes, and in the dark this isozyme acts in glycolysis to oxidize glyceraldehyde 3-phosphate. Both glyceraldehyde 3-phosphate dehydrogenase isozymes, like all enzymes, catalyze the reaction in both directions.

One molecule of glyceraldehyde 3-phosphate is the net product of the carbon assimilation pathway. The other five triose phosphate molecules (15 carbons) are rearranged in steps ① to ⑨ of Figure 20–10 to form three molecules of ribulose 1,5-bisphosphate (15 carbons). The last step in this conversion requires one ATP per ribulose 1,5-bisphosphate, or a total of three ATP. Thus, in summary, for every molecule of triose phosphate produced by photosynthetic CO_2 assimilation, six NADPH and nine ATP are required.

NADPH and ATP are produced in the light-dependent reactions of photosynthesis in about the same ratio (2:3) as they are consumed in the Calvin cycle. Nine ATP molecules are converted to ADP and phosphate in the generation of a molecule of triose phosphate; eight of the phosphates are released as P_i and combined with eight ADP to regenerate ATP. The ninth phosphate is incorporated into the triose phosphate itself. To convert the ninth ADP to ATP, a molecule of P_i must be imported from the cytosol, as we shall see.

In the dark, the production of ATP and NADPH by photophosphorylation, and the incorporation of CO_2 into triose phosphate (by the so-called dark reactions), cease. The "dark reactions" of photosynthesis were so named to distinguish them from the *primary* light-driven reactions of electron transfer to $NADP^+$ and synthesis of ATP, described in Chapter 19. They do not, in

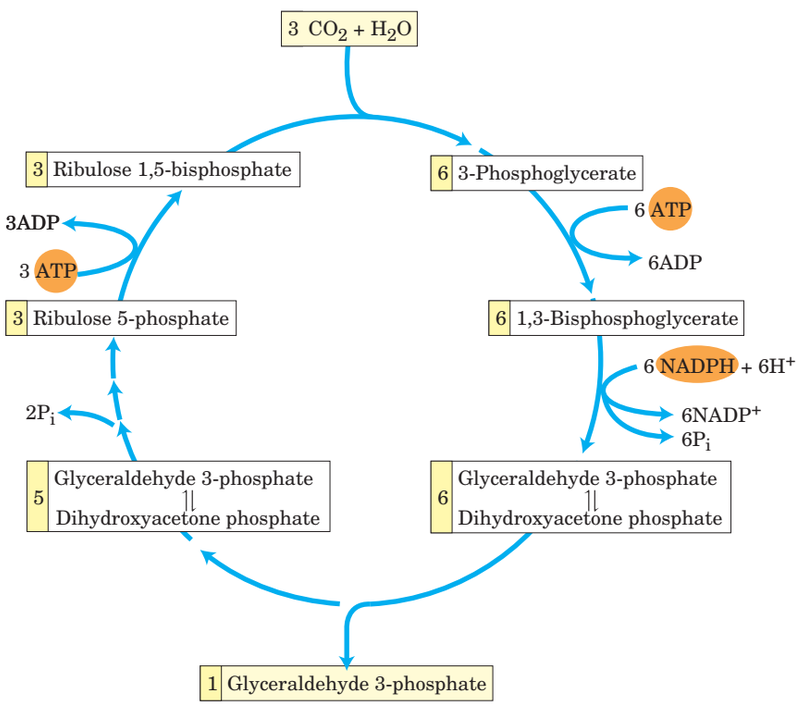

FIGURE 20–14 Stoichiometry of CO_2 assimilation in the Calvin cycle. For every three CO_2 molecules fixed, one molecule of triose phosphate (glyceraldehyde 3-phosphate) is produced and nine ATP and six NADPH are consumed.

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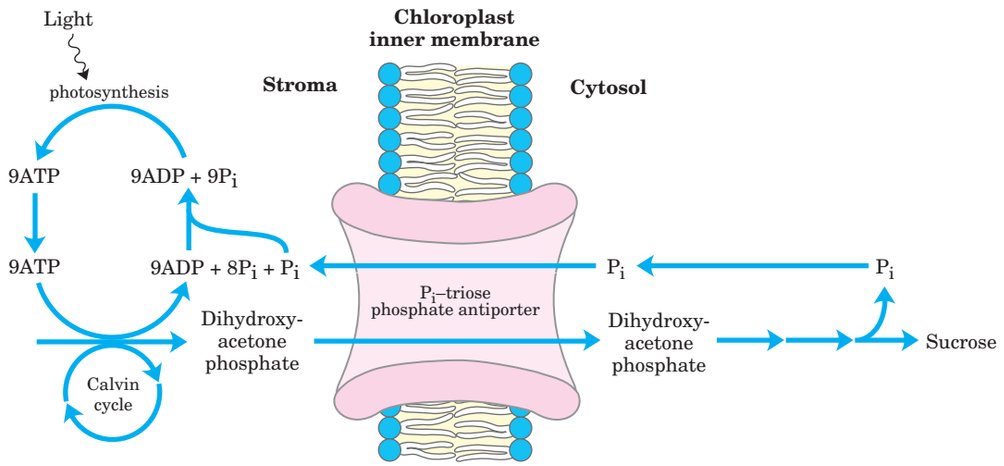

FIGURE 20–15 The P_i–triose phosphate antiport system of the inner chloroplast membrane. This transporter facilitates the exchange of cytosolic P_i for stromal dihydroxyacetone phosphate. The products of photosynthetic carbon assimilation are thus moved into the cytosol where they serve as a starting point for sucrose biosynthesis, and P_i required for photophosphorylation is moved into the stroma. This same antiporter can transport 3-phosphoglycerate and acts in the shuttle for exporting ATP and reducing equivalents (see Fig. 20–16).

fact, occur at significant rates in the dark and are thus more appropriately called the **carbon-assimilation reactions.** Later in this section we describe the regulatory mechanisms that turn on carbon assimilation in the light and turn it off in the dark.

The chloroplast stroma contains all the enzymes necessary to convert the triose phosphates produced by CO_2 assimilation (glyceraldehyde 3-phosphate and dihydroxyacetone phosphate) to starch, which is temporarily stored in the chloroplast as insoluble granules. Aldolase condenses the trioses to fructose 1,6-bisphosphate; fructose 1,6-bisphosphatase produces fructose 6-phosphate; phosphohexose isomerase yields glucose 6-phosphate; and phosphoglucomutase produces glucose 1-phosphate, the starting material for starch synthesis (see Section 20.3).

All the reactions of the Calvin cycle except those catalyzed by rubisco, sedoheptulose 1,7-bisphosphatase, and ribulose 5-phosphate kinase also take place in animal tissues. Lacking these three enzymes, animals cannot carry out net conversion of CO_2 to glucose.

A Transport System Exports Triose Phosphates from the Chloroplast and Imports Phosphate

The inner chloroplast membrane is impermeable to most phosphorylated compounds, including fructose 6-phosphate, glucose 6-phosphate, and fructose 1,6-bisphosphate. It does, however, have a specific antiporter that catalyzes the one-for-one exchange of P_i with a triose phosphate, either dihydroxyacetone phosphate or 3-phosphoglycerate (Fig. 20–15; see also Fig. 20–9). This antiporter simultaneously moves P_i into the chloroplast, where it is used in photophosphorylation, and moves triose phosphate into the cytosol, where it can be used to synthesize sucrose, the form in which the fixed carbon is transported to distant plant tissues.

Sucrose synthesis in the cytosol and starch synthesis in the chloroplast are the major pathways by which the excess triose phosphate from photosynthesis is "harvested." Sucrose synthesis (described below) releases four P_i molecules from the four triose phosphates required to make sucrose. For every molecule of triose phosphate removed from the chloroplast, one P_i is transported into the chloroplast, providing the ninth P_i mentioned above, to be used in regenerating ATP. If this exchange were blocked, triose phosphate synthesis would quickly deplete the available P_i in the chloroplast, slowing ATP synthesis and suppressing assimilation of CO_2 into starch.

The P_i–triose phosphate antiport system serves one additional function. ATP and reducing power are needed in the cytosol for a variety of synthetic and energy-requiring reactions. These requirements are met to an as yet undetermined degree by mitochondria, but a second potential source of energy is the ATP and NADPH generated in the chloroplast stroma during the light reactions. However, neither ATP nor NADPH can cross the chloroplast membrane. The P_i–triose phosphate antiport system has the indirect effect of moving ATP

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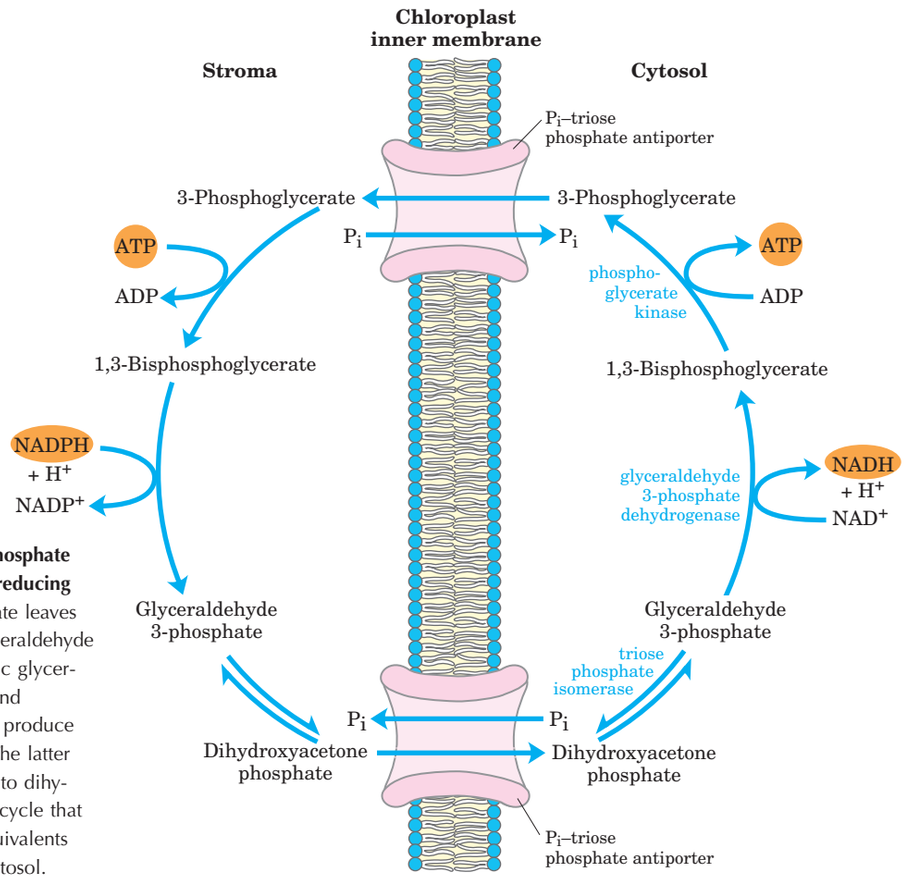

FIGURE 20–16 Role of the P_i–triose phosphate antiporter in the transport of ATP and reducing equivalents. Dihydroxyacetone phosphate leaves the chloroplast and is converted to glyceraldehyde 3-phosphate in the cytosol. The cytosolic glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase reactions then produce NADH, ATP, and 3-phosphoglycerate. The latter reenters the chloroplast and is reduced to dihydroxyacetone phosphate, completing a cycle that effectively moves ATP and reducing equivalents (NADPH/NADH) from chloroplast to cytosol.

equivalents and reducing equivalents from the chloroplast to the cytosol (Fig. 20–16). Dihydroxyacetone phosphate formed in the stroma is transported to the cytosol, where it is converted by glycolytic enzymes to 3-phosphoglycerate, generating ATP and NADH. 3-Phosphoglycerate reenters the chloroplast, completing the cycle.

Four Enzymes of the Calvin Cycle Are Indirectly Activated by Light

The reductive assimilation of CO_2 requires a lot of ATP and NADPH, and their stromal concentrations increase when chloroplasts are illuminated (Fig. 20–17). The light-induced transport of protons across the thylakoid membrane (Chapter 19) also increases the stromal pH from about 7 to about 8, and it is accompanied by a flow of Mg^{2+} from the thylakoid compartment into the stroma, raising the [Mg^{2+}] from 1 to 3 mM to 3 to 6 mM. Several stromal enzymes have evolved to take advantage of these light-induced conditions, which signal the availability of ATP and NADPH: the enzymes are more active in an alkaline environment and at high [Mg^{2+}]. For example, activation of rubisco by formation of the carbamoyllysine is faster at alkaline pH, and high stromal [Mg^{2+}] favors formation of the enzyme's active Mg^{2+} complex. Fructose 1,6-bisphosphatase requires Mg^{2+} and is very dependent on pH (Fig. 20–18); its activity increases more than 100-fold when pH and [Mg^{2+}] rise during chloroplast illumination.

Four Calvin cycle enzymes are subject to a special type of regulation by light. Ribulose 5-phosphate kinase, fructose 1,6-bisphosphatase, sedoheptulose 1,7-bisphosphatase, and glyceraldehyde 3-phosphate dehydrogenase are activated by light-driven reduction of disulfide bonds between two Cys residues critical to their catalytic activities. When these Cys residues are disulfide-bonded (oxidized), the enzymes are inactive; this is the normal situation in the dark. With illumination, electrons flow from photosystem I to ferredoxin (see Fig. 19–49), which passes electrons to a small, soluble, disulfide-containing protein called **thioredoxin** (Fig. 20–19), in a reaction catalyzed by **ferredoxin-thioredoxin reductase**. Reduced thioredoxin donates electrons for the reduction of the disulfide bonds of the light-activated enzymes, and these reductive cleavage reactions are accompanied by conformational changes that increase enzyme activities. At nightfall, the Cys residues in the

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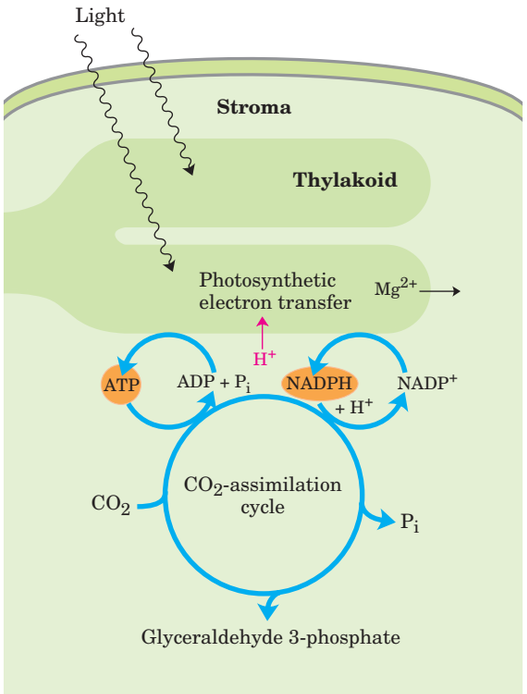

FIGURE 20–17 Source of ATP and NADPH. ATP and NADPH produced by the light reactions are essential substrates for the reduction of CO_2. The photosynthetic reactions that produce ATP and NADPH are accompanied by movement of protons (red) from the stroma into the thylakoid, creating alkaline conditions in the stroma. Magnesium ions pass from the thylakoid into the stroma, increasing the stromal [Mg^{2+}].

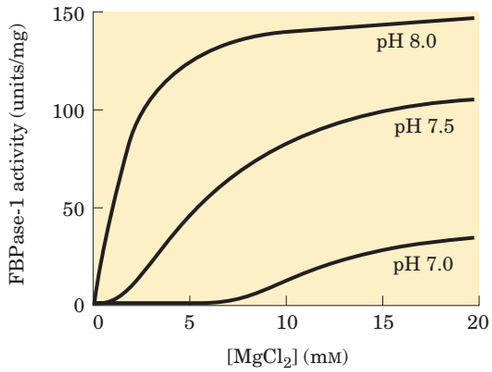

FIGURE 20–18 Activation of chloroplast fructose 1,6-bisphosphatase. Reduced fructose 1,6-bisphosphatase (FBPase-1) is activated by light and by the combination of high pH and high [Mg^{2+}] in the stroma, both of which are produced by illumination.

four enzymes are reoxidized to their disulfide forms, the enzymes are inactivated, and ATP is not expended in CO_2 assimilation. Instead, starch synthesized and stored during the daytime is degraded to fuel glycolysis at night.

Glucose 6-phosphate dehydrogenase, the first enzyme in the *oxidative* pentose phosphate pathway, is also regulated by this light-driven reduction mechanism, but in the opposite sense. During the day, when photosynthesis produces plenty of NADPH, this enzyme is not needed for NADPH production. Reduction of a critical disulfide bond by electrons from ferredoxin *inactivates* the enzyme.

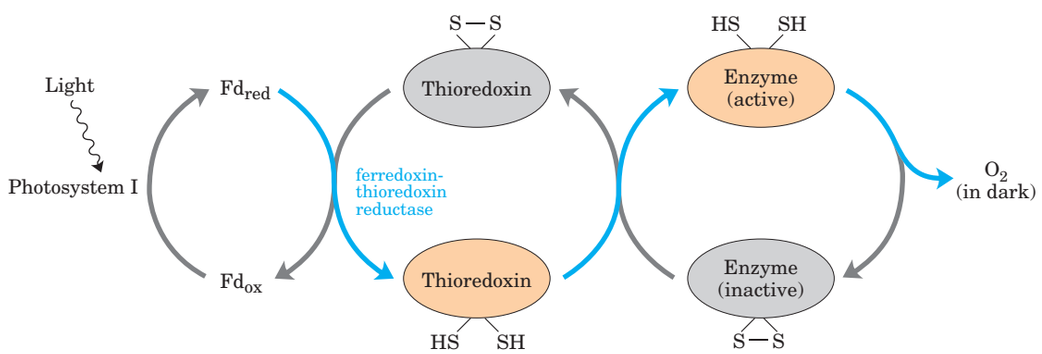

FIGURE 20–19 Light activation of several enzymes of the Calvin cycle. The light activation is mediated by thioredoxin, a small, disulfide-containing protein. In the light, thioredoxin is reduced by electrons moving from photosystem I through ferredoxin (Fd) (blue arrows), then thioredoxin reduces critical disulfide bonds in each of the enzymes sedoheptulose 1,7-bisphosphatase, fructose 1,6-bisphosphatase, ribulose 5-phosphate kinase, and glyceraldehye 3-phosphate dehydrogenase, activating these enzymes. In the dark, the —SH groups undergo reoxidation to disulfides, inactivating the enzymes.

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SUMMARY 20.1 Photosynthetic Carbohydrate Synthesis

- Photosynthesis in vascular plants takes place in chloroplasts. In the CO_2-assimilating reactions (the Calvin cycle), ATP and NADPH are used to reduce CO_2 to triose phosphates. These reactions occur in three stages: the fixation reaction itself, catalyzed by rubisco; reduction of the resulting 3-phosphoglycerate to glyceraldehyde 3-phosphate; and regeneration of ribulose 1,5-bisphosphate from triose phosphates.
- Rubisco condenses CO_2 with ribulose 1,5-bisphosphate, forming an unstable hexose bisphosphate that splits into two molecules of 3-phosphoglycerate. Rubisco is activated by covalent modification (carbamoylation of Lys^{201}) catalyzed by rubisco activase and is inhibited by a natural transition-state analog, whose concentration rises in the dark and falls during daylight.
- Stromal isozymes of the glycolytic enzymes catalyze reduction of 3-phosphoglycerate to glyceraldehyde 3-phosphate; each molecule reduced requires one ATP and one NADPH.
- Stromal enzymes, including transketolase and transaldolase, rearrange the carbon skeletons of triose phosphates, generating intermediates of three, four, five, six, and seven carbons and eventually yielding pentose phosphates. The pentose phosphates are converted to ribulose 5-phosphate, then phosphorylated to ribulose 1,5-bisphosphate to complete the Calvin cycle.
- The cost of fixing three CO_2 into one triose phosphate is nine ATP and six NADPH, which are provided by the light-dependent reactions of photosynthesis.
- An antiporter in the inner chloroplast membrane exchanges P_i in the cytosol for 3-phosphoglycerate or dihydroxyacetone phosphate produced by CO_2 assimilation in the stroma. Oxidation of dihydroxyacetone phosphate in the cytosol generates ATP and NADH, thus moving ATP and reducing equivalents from the chloroplast to the cytosol.
- Four enzymes of the Calvin cycle are activated indirectly by light and are inactive in the dark, so that hexose synthesis does not compete with glycolysis—which is required to provide energy in the dark.

20.2 Photorespiration and the C_4 and CAM Pathways

As we have seen, photosynthetic cells produce O_2 (by the splitting of H_2O) during the light-driven reactions (Chapter 19) and use CO_2 during the light-independent processes (described above), so the net gaseous change during photosynthesis is the uptake of CO_2 and release of O_2:

$$CO_2 + H_2O \longrightarrow O_2 + (CH_2O)$$

In the dark, plants also carry out **mitochondrial respiration,** the oxidation of substrates to CO_2 and the conversion of O_2 to H_2O. And there is another process in plants that, like mitochondrial respiration, consumes O_2 and produces CO_2 and, like photosynthesis, is driven by light. This process, **photorespiration,** is a costly side reaction of photosynthesis, a result of the lack of specificity of the enzyme rubisco. In this section we describe this side reaction and the strategies plants use to minimize its metabolic consequences.

Photorespiration Results from Rubisco's Oxygenase Activity

Rubisco is not absolutely specific for CO_2 as a substrate. Molecular oxygen (O_2) competes with CO_2 at the active site, and about once in every three or four turnovers, rubisco catalyzes the condensation of O_2 with ribulose 1,5-bisphosphate to form 3-phosphoglycerate and **2-phosphoglycolate** (Fig. 20–20), a metabolically useless product. This is the oxygenase activity referred to in the full name of the enzyme: ribulose 1,5-bisphosphate carboxylase/oxygenase. The reaction with O_2 results in no fixation of carbon and appears to be a net liability to the cell; salvaging the carbons from 2-phosphoglycolate (by the pathway outlined below) consumes significant amounts of cellular energy and releases some previously fixed CO_2.

Given that the reaction with oxygen is deleterious to the organism, why did the evolution of rubisco produce an active site unable to discriminate well between CO_2 and O_2? Perhaps much of this evolution occurred before the time, about 2.5 billion years ago, when production of O_2 by photosynthetic organisms started to raise the oxygen content of the atmosphere. Before that time, there was no selective pressure for rubisco to discriminate between CO_2 and O_2. The K_m for CO_2 is about 9 μM, and that for O_2 is about 350 μM. The modern atmosphere contains about 20% O_2 and only 0.04% CO_2, so an aqueous solution in equilibrium with air at room temperature contains about 250 μM O_2 and 11 μM CO_2—concentrations that allow significant O_2 "fixation" by rubisco and thus a significant waste of energy. The temperature dependence of the solubilities of O_2 and CO_2 is

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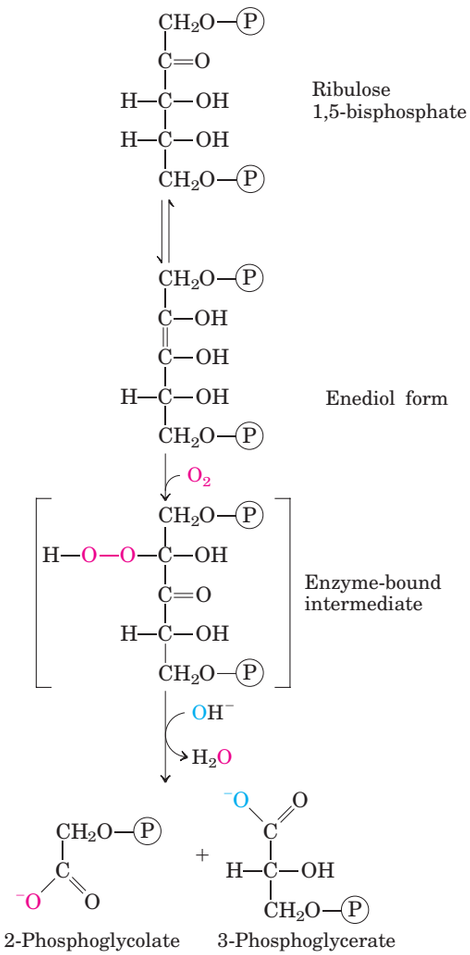

FIGURE 20–20 Oxygenase activity of rubisco. Rubisco can incorporate O_2 rather than CO_2 into ribulose 1,5-bisphosphate. The unstable intermediate thus formed splits into 2-phosphoglycolate (recycled as described in Fig. 20–21) and 3-phosphoglycerate, which can reenter the Calvin cycle.

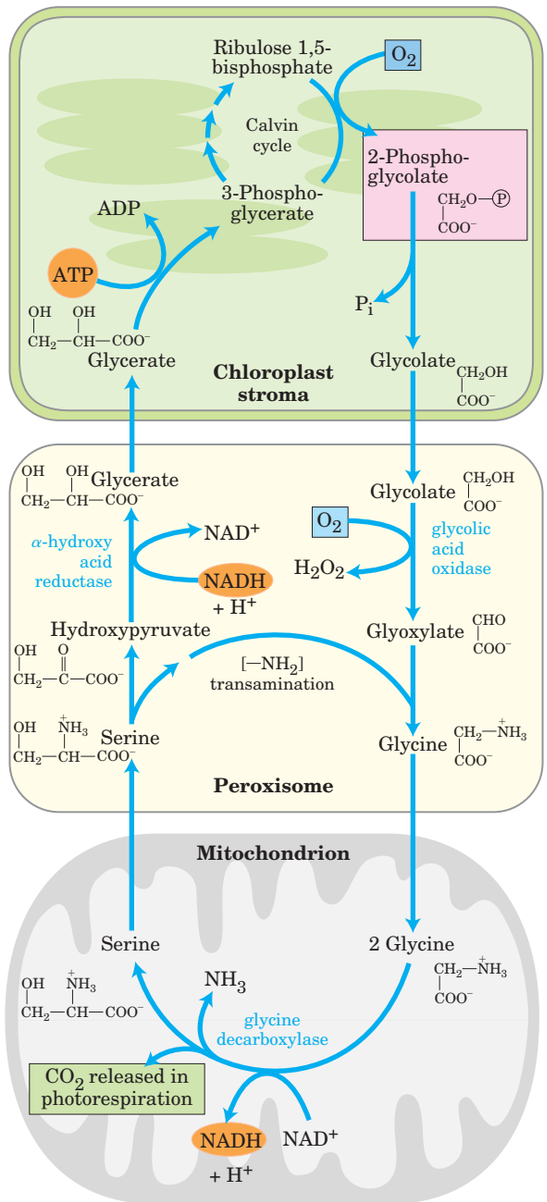

FIGURE 20–21 Glycolate pathway. This pathway, which salvages 2-phosphoglycolate (shaded pink) by its conversion to serine and eventually 3-phosphoglycerate, involves three cellular compartments. Glycolate formed by dephosphorylation of 2-phosphoglycolate in chloroplasts is oxidized to glyoxylate in peroxisomes and then transaminated to glycine. In mitochondria, two glycine molecules condense to form serine and the CO_2 released during photorespiration (shaded green). This reaction is catalyzed by glycine decarboxylase, an enzyme present at very high levels in the mitochondria of C_3 plants (see text). The serine is converted to hydroxypyruvate and then to glycerate in peroxisomes; glycerate reenters the chloroplasts to be phosphorylated, rejoining the Calvin cycle. Oxygen (shaded blue) is consumed at two steps during photorespiration.

such that at higher temperatures, the ratio of O_2 to CO_2 in solution increases. In addition, the affinity of rubisco for CO_2 decreases with increasing temperature, exacerbating its tendency to catalyze the wasteful oxygenase reaction. And as CO_2 is consumed in the assimilation reactions, the ratio of O_2 to CO_2 in the air spaces of a leaf increases, further favoring the oxygenase reaction.

The Salvage of Phosphoglycolate Is Costly

The **glycolate pathway** converts two molecules of 2-phosphoglycolate to a molecule of serine (three carbons) and a molecule of CO_2 (Fig. 20–21). In the chloroplast, a phosphatase converts 2-phosphoglycolate to glycolate, which is exported to the peroxisome. There, glycolate is

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oxidized by molecular oxygen, and the resulting aldehyde (glyoxylate) undergoes transamination to glycine. The hydrogen peroxide formed as a side product of glycolate oxidation is rendered harmless by peroxidases in the peroxisome. Glycine passes from the peroxisome to the mitochondrial matrix, where it undergoes oxidative decarboxylation by the glycine decarboxylase complex, an enzyme similar in structure and mechanism to two mitochondrial complexes we have already encountered: the pyruvate dehydrogenase complex and the α-ketoglutarate dehydrogenase complex (Chapter 16). The **glycine decarboxylase complex** oxidizes glycine to CO_2 and NH_3, with the concomitant reduction of NAD^+ to NADH and transfer of the remaining carbon from glycine to the cofactor tetrahydrofolate (Fig. 20–22). The one-carbon unit carried on tetrahydrofolate is then transferred to a second glycine by serine hydroxymethyltransferase, producing serine. The net reaction catalyzed by the glycine decarboxylase complex and serine hydroxymethyltransferase is

$$2\ \text{Glycine} + NAD^+ + H_2O \longrightarrow \text{serine} + CO_2 + NH_3 + NADH + H^+$$

The serine is converted to hydroxypyruvate, to glycerate, and finally to 3-phosphoglycerate, which is used to regenerate ribulose 1,5-bisphosphate, completing the long, expensive cycle (Fig. 20–21).

In bright sunlight, the flux through the glycolate salvage pathway can be very high, producing about five times more CO_2 than is typically produced by all the ox-

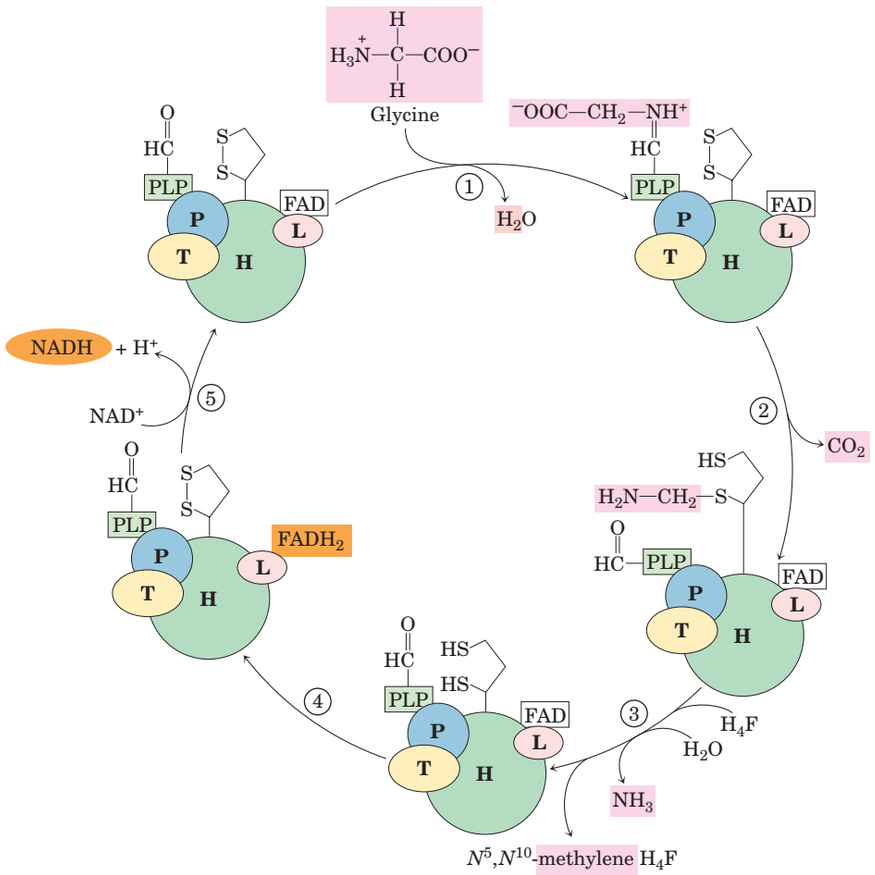

FIGURE 20–22 The glycine decarboxylase system. Glycine decarboxylase in plant mitochondria is a complex of four types of subunits, with the stoichiometry $P_4H_{27}T_9L_2$. Protein H has a covalently attached lipoic acid residue that can undergo reversible oxidation. Step ① is formation of a Schiff base between pyridoxal phosphate (PLP) and glycine, catalyzed by protein P (named for its bound PLP). In step ②, protein P catalyzes oxidative decarboxylation of glycine, releasing CO_2; the remaining methylamine group is attached to one of the —SH groups of reduced lipoic acid. ③ Protein T (which uses tetrahydrofolate (H_4F) as cofactor) now releases NH_3 from the methylamine moiety and transfers the remaining one-carbon fragment to tetrahydrofolate, producing N^5,N^{10}-methylene tetrahydrofolate. ④ Protein L oxidizes the two —SH groups of lipoic acid to a disulfide, passing electrons through FAD to NAD^+ ⑤, thus completing the cycle. The N^5,N^{10}-methylene tetrahydrofolate formed in this process is used by serine hydroxymethyltransferase to convert a molecule of glycine to serine, regenerating the tetrahydrofolate that is essential for the reaction catalyzed by protein T. The L subunit of glycine decarboxylase is identical to the dihydrolipoyl dehydrogenase (E_3) of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (see Fig. 16–6).

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idations of the citric acid cycle. To generate this large flux, mitochondria contain prodigious amounts of the glycine decarboxylase complex: the four proteins of the complex make up *half* of all the protein in the mitochondrial matrix in the leaves of pea and spinach plants! In nonphotosynthetic parts of a plant, such as potato tubers, mitochondria have very low concentrations of the glycine decarboxylase complex.

The combined activity of the rubisco oxygenase and the glycolate salvage pathway consumes O_2 and produces CO_2—hence the name **photorespiration.** This pathway is perhaps better called the **oxidative photosynthetic carbon cycle** or **C_2 cycle**, names that do not invite comparison with respiration in mitochondria. Unlike mitochondrial respiration, "photorespiration" does not conserve energy and may actually inhibit net biomass formation as much as 50%. This inefficiency has led to evolutionary adaptations in the carbon-assimilation processes, particularly in plants that have evolved in warm climates.

In C_4 Plants, CO_2 Fixation and Rubisco Activity Are Spatially Separated

In many plants that grow in the tropics (and in temperate-zone crop plants native to the tropics, such as maize, sugarcane, and sorghum) a mechanism has evolved to circumvent the problem of wasteful photorespiration. The step in which CO_2 is fixed into a three-carbon product, 3-phosphoglycerate, is preceded by several steps, one of which is temporary fixation of CO_2 into a four-carbon compound. Plants that use this process are referred to as **C_4 plants,** and the assimilation process as **C_4 metabolism** or the **C_4 pathway.** Plants that use the carbon-assimilation method we have described thus far, in which the *first step* is reaction of CO_2 with ribulose 1,5-bisphosphate to form 3-phosphoglycerate, are called **C_3 plants.**

The C_4 plants, which typically grow at high light intensity and high temperatures, have several important characteristics: high photosynthetic rates, high growth rates, low photorespiration rates, low rates of water loss, and a specialized leaf structure. Photosynthesis in the leaves of C_4 plants involves two cell types: mesophyll and bundle-sheath cells (Fig. 20–23a). There are three variants of C_4 metabolism, worked out in the 1960s by Marshall Hatch and Rodger Slack (Fig. 20–23b).

In plants of tropical origin, the first intermediate into which $^{14}CO_2$ is fixed is oxaloacetate, a four-carbon compound. This reaction, which occurs in the cytosol of leaf mesophyll cells, is catalyzed by **phosphoenolpyruvate carboxylase,** for which the substrate is HCO_3^-, not CO_2. The oxaloacetate thus formed is either reduced to malate at the expense of NADPH (as shown in Fig. 20–23b) or converted to aspartate by transamination:

Oxaloacetate + α-amino acid ⟶ L-aspartate + α-keto acid

The malate or aspartate formed in the mesophyll cells then passes into neighboring bundle-sheath cells through plasmodesmata, protein-lined channels that connect two plant cells and provide a path for movement of metabolites and even small proteins between cells. In the bundle-sheath cells, malate is oxidized and decarboxylated to yield pyruvate and CO_2 by the action of **malic enzyme,** reducing $NADP^+$. In plants that use aspartate as the CO_2 carrier, aspartate arriving in bundle-sheath cells is transaminated to form oxaloacetate and reduced to malate, then the CO_2 is released by malic enzyme or PEP carboxykinase. As labeling experiments show, the free CO_2 released in the bundle-sheath cells is the same CO_2 molecule originally fixed into oxaloacetate in the mesophyll cells. This CO_2 is now fixed again, this time by rubisco, in exactly the same reaction that occurs in C_3 plants: incorporation of CO_2 into C-1 of 3-phosphoglycerate.

The pyruvate formed by decarboxylation of malate in bundle-sheath cells is transferred back to the mesophyll cells, where it is converted to PEP by an unusual enzymatic reaction catalyzed by **pyruvate phosphate dikinase** (Fig. 20–23b). This enzyme is called a dikinase because two different molecules are simultaneously phosphorylated by one molecule of ATP: pyruvate to PEP, and phosphate to pyrophosphate. The pyrophosphate is subsequently hydrolyzed to phosphate, so two high-energy phosphate groups of ATP are used in regenerating PEP. The PEP is now ready to receive another molecule of CO_2 in the mesophyll cell.

The PEP carboxylase of mesophyll cells has a high affinity for HCO_3^- (which is favored relative to CO_2 in aqueous solution and can fix CO_2 more efficiently than can rubisco). Unlike rubisco, it does not use O_2 as an alternative substrate, so there is no competition between CO_2 and O_2. The PEP carboxylase reaction, then, serves to fix and concentrate CO_2 in the form of malate. Release of CO_2 from malate in the bundle-sheath cells yields a sufficiently high local concentration of CO_2 for rubisco to function near its maximal rate, and for suppression of the enzyme's oxygenase activity.

Once CO_2 is fixed into 3-phosphoglycerate in the bundle-sheath cells, the other reactions of the Calvin cycle take place exactly as described earlier. Thus in C_4 plants, mesophyll cells carry out CO_2 assimilation by the C_4 pathway and bundle-sheath cells synthesize starch and sucrose by the C_3 pathway.

Three enzymes of the C_4 pathway are regulated by light, becoming more active in daylight. Malate dehydrogenase is activated by the thioredoxin-dependent reduction mechanism shown in Figure 20–19; PEP carboxylase is activated by phosphorylation of a Ser residue; and pyruvate phosphate dikinase is activated by dephosphorylation. In the latter two cases, the details of how light effects phosphorylation or dephosphorylation are not known.

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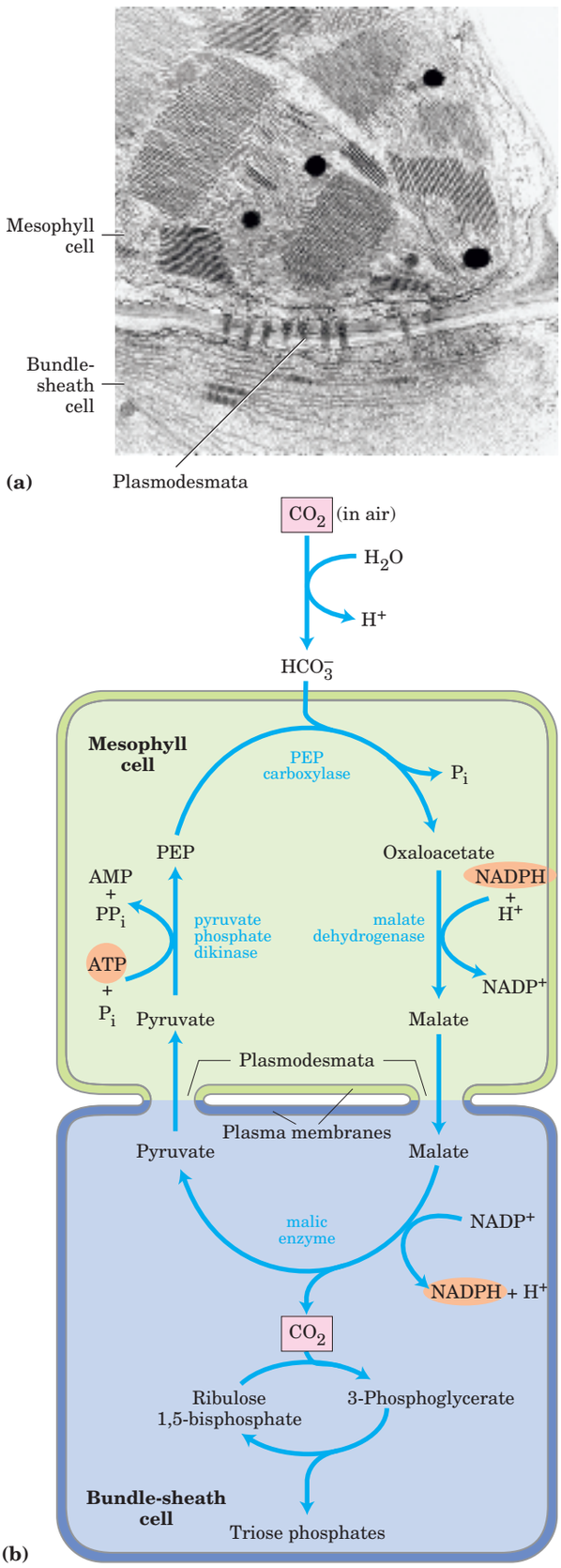

The pathway of CO_2 assimilation has a greater energy cost in C_4 plants than in C_3 plants. For each molecule of CO_2 assimilated in the C_4 pathway, a molecule of PEP must be regenerated at the expense of two high-energy phosphate groups of ATP. Thus C_4 plants need five ATP molecules to assimilate one molecule of CO_2, whereas C_3 plants need only three (nine per triose phosphate). As the temperature increases (and the affinity of rubisco for CO_2 decreases, as noted above), a point is reached (at about 28 to 30 °C) at which the gain in efficiency from the elimination of photorespiration more than compensates for this energetic cost. C_4 plants (crabgrass, for example) outgrow most C_3 plants during the summer, as any experienced gardener can attest.

In CAM Plants, CO_2 Capture and Rubisco Action Are Temporally Separated

Succulent plants such as cactus and pineapple, which are native to very hot, very dry environments, have another variation on photosynthetic CO_2 fixation, which reduces loss of water vapor through the pores (stomata) by which CO_2 and O_2 must enter leaf tissue. Instead of separating the initial trapping of CO_2 and its fixation by rubisco across space (as do the C_4 plants), they separate these two events over time. At night, when the air is cooler and moister, the stomata open to allow entry of CO_2, which is then fixed into oxaloacetate by PEP carboxylase. The oxaloacetate is reduced to malate and stored in the vacuoles, to protect cytosolic and plastid enzymes from the low pH produced by malic acid dissociation. During the day the stomata close, preventing the water loss that would result from high daytime temperatures, and the CO_2 trapped overnight in malate is released as CO_2 by the NADP-linked malic enzyme. This CO_2 is now assimilated by the action of rubisco and the Calvin cycle enzymes. Because this method of CO_2 fixation was first discovered in stonecrops, perennial flowering plants of the family *Crassulaceae*, it is called *c*rassulacean *a*cid *m*etabolism, and the plants are called **CAM plants.**

FIGURE 20–23 Carbon assimilation in C_4 plants. The C_4 pathway, involving mesophyll cells and bundle-sheath cells, predominates in plants of tropical origin. **(a)** Electron micrograph showing chloroplasts of adjacent mesophyll and bundle-sheath cells. The bundle-sheath cell contains starch granules. Plasmodesmata connecting the two cells are visible. **(b)** The C_4 pathway of CO_2 assimilation, which occurs through a four-carbon intermediate.

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SUMMARY 20.2 Photorespiration and the C_4 and CAM Pathways

- When rubisco uses O_2 rather than CO_2 as substrate, the 2-phosphoglycolate so formed is disposed of in an oxygen-dependent pathway. The result is increased consumption of O_2—photorespiration or, more accurately, the oxidative photosynthetic carbon cycle or C_2 cycle. The 2-phosphoglycolate is converted to glyoxylate, to glycine, and then to serine in a pathway that involves enzymes in the chloroplast stroma, the peroxisome, and the mitochondrion.
- In C_4 plants, the carbon-assimilation pathway minimizes photorespiration: CO_2 is first fixed in mesophyll cells into a four-carbon compound, which passes into bundle-sheath cells and releases CO_2 in high concentrations. The released CO_2 is fixed by rubisco, and the remaining reactions of the Calvin cycle occur as in C_3 plants.
- In CAM plants, CO_2 is fixed into malate in the dark and stored in vacuoles until daylight, when the stomata are closed (minimizing water loss) and malate serves as a source of CO_2 for rubisco.

20.3 Biosynthesis of Starch and Sucrose

During active photosynthesis in bright light, a plant leaf produces more carbohydrate (as triose phosphates) than it needs for generating energy or synthesizing precursors. The excess is converted to sucrose and transported to other parts of the plant, to be used as fuel or stored. In most plants, starch is the main storage form, but in a few plants, such as sugar beet and sugarcane, sucrose is the primary storage form. The synthesis of sucrose and starch occurs in different cellular compartments (cytosol and plastids, respectively), and these processes are coordinated by a variety of regulatory mechanisms that respond to changes in light level and photosynthetic rate.

ADP-Glucose Is the Substrate for Starch Synthesis in Plant Plastids and for Glycogen Synthesis in Bacteria

Starch, like glycogen, is a high molecular weight polymer of D-glucose in (α1→4) linkage. It is synthesized in chloroplasts for temporary storage as one of the stable end products of photosynthesis, and for long-term storage it is synthesized in the amyloplasts of the nonphotosynthetic parts of plants—seeds, roots, and tubers (underground stems).

The mechanism of glucose activation in starch synthesis is similar to that in glycogen synthesis. An activated **nucleotide sugar,** in this case **ADP-glucose,** is formed by condensation of glucose 1-phosphate with ATP in a reaction made essentially irreversible by the presence in plastids of inorganic pyrophosphatase (p. 502). **Starch synthase** then transfers glucose residues from ADP-glucose to preexisting starch molecules. Although it has generally been assumed that glucose is added to the *nonreducing* end of starch, as in glycogen synthesis (see Fig. 15–8), evidence now suggests that starch synthase has two equivalent active sites that alternate in inserting a glucosyl residue onto the *reducing* end of the growing chain. This end remains covalently attached to the enzyme, first at one active site, then at the other (Fig. 20–24). Attachment to one active site effectively activates the reducing end of the growing chain for nucleophilic displacement of the enzyme by the attacking C-4 hydroxyl of a glucosyl moiety bound to the other active site, forming the (α1→4) linkage characteristic of starch.

The amylose of starch is unbranched, but amylopectin has numerous (α1→6)-linked branches (see Fig. 7–15). Chloroplasts contain a branching enzyme, similar to glycogen-branching enzyme (see Fig. 15–9), that introduces the (α1→6) branches of amylopectin. Taking into account the hydrolysis by inorganic pyrophosphatase of the PP_i produced during ADP-glucose synthesis, the overall reaction for starch formation from glucose 1-phosphate is

$$\text{Starch}_n + \text{glucose 1-phosphate} + \text{ATP} \longrightarrow \text{starch}_{n+1} + \text{ADP} + 2\text{P}_i \qquad \Delta G'^\circ = -50 \text{ kJ/mol}$$

Starch synthesis is regulated at the level of ADP-glucose formation, as discussed below.

Many types of bacteria store carbohydrate in the form of glycogen (essentially highly branched starch), which they synthesize in a reaction analogous to that catalyzed by glycogen synthase in animals. Bacteria, like plant plastids, use ADP-glucose as the activated form of glucose, whereas animal cells use UDP-glucose. Again, the similarity between plastid and bacterial metabolism is consistent with the endosymbiont hypothesis for the origin of organelles (see Fig. 1–36).

UDP-Glucose Is the Substrate for Sucrose Synthesis in the Cytosol of Leaf Cells

Most of the triose phosphate generated by CO_2 fixation in plants is converted to sucrose (Fig. 20–25) or starch. In the course of evolution, sucrose may have been selected as the transport form of carbon because of its unusual linkage between the anomeric C-1 of glucose and the anomeric C-2 of fructose. This bond is not hydrolyzed by amylases or other common carbohydrate-cleaving

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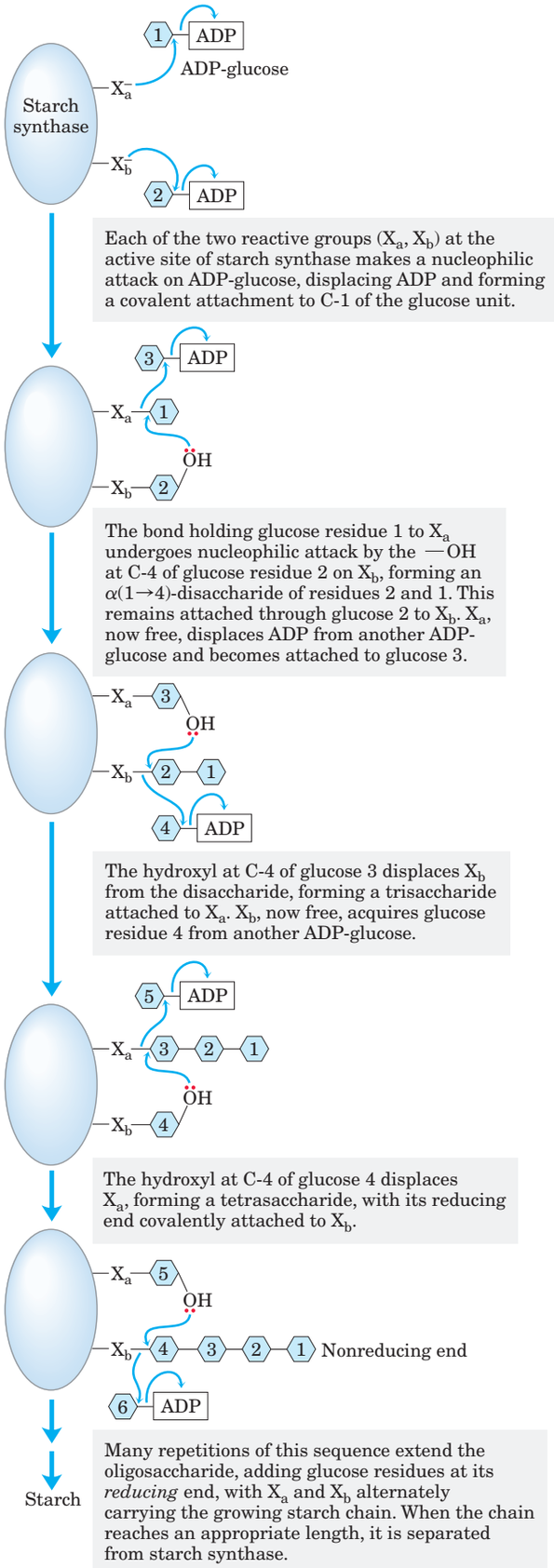

FIGURE 20–24 Starch synthesis. Starch synthesis proceeds by a two-site insertion mechanism, with ADP-glucose as the initial glucosyl donor. The two identical active sites on starch synthase alternate in displacing the growing chain from each other, and new glucosyl units are inserted at the reducing end of the growing chain.

enzymes, and the unavailability of the anomeric carbons prevents sucrose from reacting nonenzymatically (as does glucose) with amino acids and proteins.

Sucrose is synthesized in the cytosol, beginning with dihydroxyacetone phosphate and glyceraldehyde 3-phosphate exported from the chloroplast. After condensation of two triose phosphates to form fructose 1,6-bisphosphate (catalyzed by aldolase), hydrolysis by fructose 1,6-bisphosphatase yields fructose 6-phosphate. **Sucrose 6-phosphate synthase** then catalyzes the reaction of fructose 6-phosphate with **UDP-glucose** to form **sucrose 6-phosphate** (Fig. 20–25). Finally, **sucrose 6-phosphate phosphatase** removes the phosphate group, making sucrose available for export to other tissues. The reaction catalyzed by sucrose 6-phosphate synthase is a low-energy process ($\Delta G'^\circ = -5.7$ kJ/mol), but the hydrolysis of sucrose 6-phosphate to sucrose is sufficiently exergonic ($\Delta G'^\circ = -16.5$ kJ/mol) to make the overall synthesis of sucrose essentially irreversible. Sucrose synthesis is regulated and closely coordinated with starch synthesis, as we shall see.

One remarkable difference between the cells of plants and animals is the absence in the plant cell cytosol of the enzyme inorganic pyrophosphatase, which catalyzes the reaction

$$PP_i + H_2O \longrightarrow 2P_i \qquad \Delta G'^\circ = -19.2 \text{ kJ/mol}$$

For many biosynthetic reactions that liberate PP_i, pyrophosphatase activity makes the process more favorable energetically, tending to make these reactions irreversible. In plants, this enzyme is present in plastids but absent from the cytosol. As a result, the cytosol of leaf cells contains a substantial concentration of PP_i—enough (~0.3 mM) to make reactions such as that catalyzed by UDP-glucose pyrophosphorylase (Fig. 15–7) readily reversible. Recall from Chapter 14 (p. 527) that the cytosolic isozyme of phosphofructokinase in plants uses PP_i, not ATP, as the phosphoryl donor.

Conversion of Triose Phosphates to Sucrose and Starch Is Tightly Regulated

Triose phosphates produced by the Calvin cycle in bright sunlight, as we have noted, may be stored temporarily in the chloroplast as starch, or converted to sucrose and exported to nonphotosynthetic parts of the plant, or both. The balance between the two processes is tightly regulated, and both must be coordinated with the rate of carbon fixation. Five-sixths of the triose

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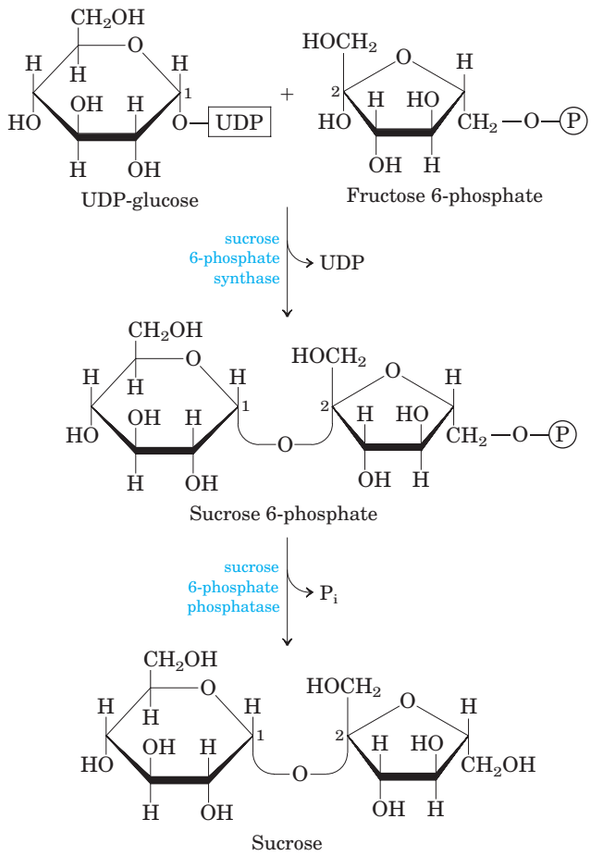

FIGURE 20–25 Sucrose synthesis. Sucrose is synthesized from UDP-glucose and fructose 6-phosphate, which are synthesized from triose phosphates in the plant cell cytosol by pathways shown in Figures 15–7 and 20–9. The sucrose 6-phosphate synthase of most plant species is allosterically regulated by glucose 6-phosphate and P_i.

phosphate formed in the Calvin cycle must be recycled to ribulose 1,5-bisphosphate (Fig. 20–14); if more than one-sixth of the triose phosphate is drawn out of the cycle to make sucrose and starch, the cycle will slow or stop. However, *insufficient* conversion of triose phosphate to starch or sucrose would tie up phosphate, leaving a chloroplast deficient in P_i, which is also essential for operation of the Calvin cycle.

The flow of triose phosphates into sucrose is regulated by the activity of fructose 1,6-bisphosphatase (FBPase-1) and the enzyme that effectively reverses its action, PP_i-dependent phosphofructokinase (PP-PFK-1; p. 527). These enzymes are therefore critical points for determining the fate of triose phosphates produced by photosynthesis. Both enzymes are regulated by **fructose 2,6-bisphosphate (F2,6BP),** which inhibits FBPase-1 and stimulates PP-PFK-1. In vascular plants, the concentration of F2,6BP varies inversely with the rate of photosynthesis (Fig. 20–26). Phosphofructokinase-2, responsible for F2,6BP synthesis, is inhibited by dihydroxyacetone phosphate or 3-phosphoglycerate and stimulated by fructose 6-phosphate and P_i. During active photosynthesis, dihydroxyacetone phosphate is produced and P_i is consumed, resulting in inhibition of PFK-2 and lowered concentrations of F2,6BP. This

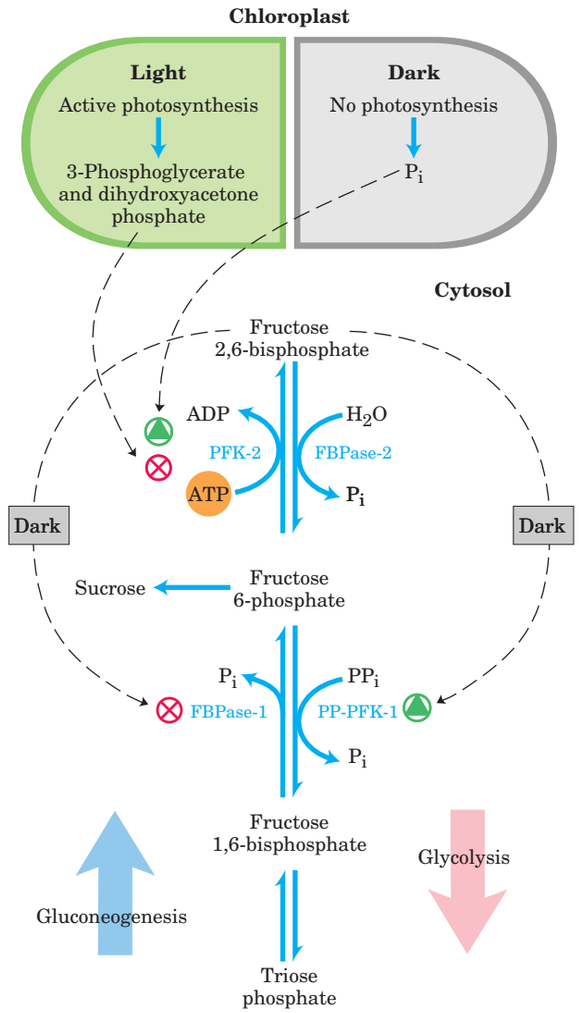

FIGURE 20–26 Fructose 2,6-bisphosphate as regulator of sucrose synthesis. The concentration of the allosteric regulator fructose 2,6-bisphosphate in plant cells is regulated by the products of photosynthetic carbon assimilation and by P_i. Dihydroxyacetone phosphate and 3-phosphoglycerate produced by CO_2 assimilation inhibit phosphofructokinase-2 (PFK-2), the enzyme that synthesizes the regulator; P_i stimulates PFK-2. The concentration of the regulator is therefore inversely proportional to the rate of photosynthesis. In the dark, the concentration of fructose 2,6-bisphosphate increases and stimulates the glycolytic enzyme PP_i-dependent phosphofructokinase-1 (PP-PFK-1), while inhibiting the gluconeogenic enzyme fructose 1,6-bisphosphatase (FBPase-1). When photosynthesis is active (in the light), the concentration of the regulator drops and the synthesis of fructose 6-phosphate and sucrose is favored.

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favors greater flux of triose phosphate into fructose 6-phosphate formation and sucrose synthesis. With this regulatory system, sucrose synthesis occurs when the level of triose phosphate produced by the Calvin cycle exceeds that needed to maintain the operation of the cycle.

Sucrose synthesis is also regulated at the level of sucrose 6-phosphate synthase, which is allosterically activated by glucose 6-phosphate and inhibited by P_i. This enzyme is further regulated by phosphorylation and dephosphorylation; a protein kinase phosphorylates the enzyme on a specific Ser residue, making it less active, and a phosphatase reverses this inactivation by removing the phosphate (Fig. 20–27). Inhibition of the kinase by glucose 6-phosphate, and of the phosphatase by P_i, strengthens the effects of these two compounds on sucrose synthesis. When hexose phosphates are abundant, sucrose 6-phosphate synthase is activated by glucose 6-phosphate; when P_i is elevated (as when photosynthesis is slow), sucrose synthesis is slowed. During active photosynthesis, triose phosphates are converted to fructose 6-phosphate, which is rapidly equilibrated with glucose 6-phosphate by phosphohexose isomerase. Because the equilibrium lies far toward glucose 6-phosphate, as soon as fructose 6-phosphate accumulates, the level of glucose 6-phosphate rises and sucrose synthesis is stimulated.

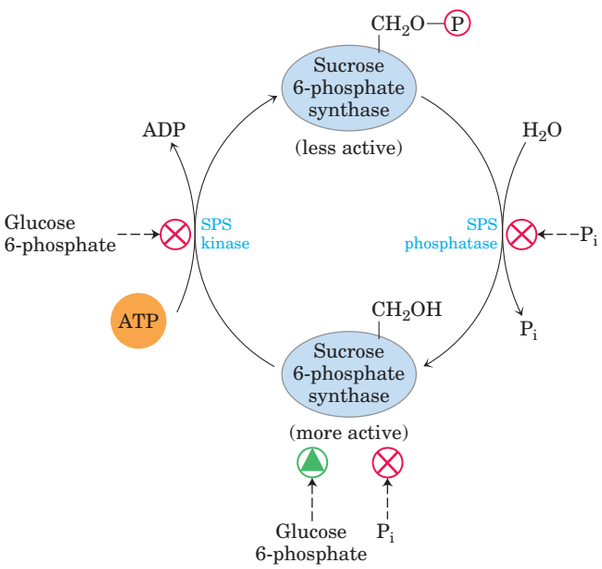

FIGURE 20–27 Regulation of sucrose phosphate synthase by phosphorylation. A protein kinase (SPS kinase) specific for sucrose phosphate synthase (SPS) phosphorylates a Ser residue in SPS, inactivating it; a specific phosphatase (SPS phosphatase) reverses this inhibition. The kinase is inhibited allosterically by glucose 6-phosphate, which also activates SPS allosterically. The phosphatase is inhibited by Pi, which also inhibits SPS directly. Thus when the concentration of glucose 6-phosphate is high as a result of active photosynthesis, SPS is activated and produces sucrose phosphate. A high P_i concentration, which occurs when photosynthetic conversion of ADP to ATP is slow, inhibits sucrose phosphate synthesis.

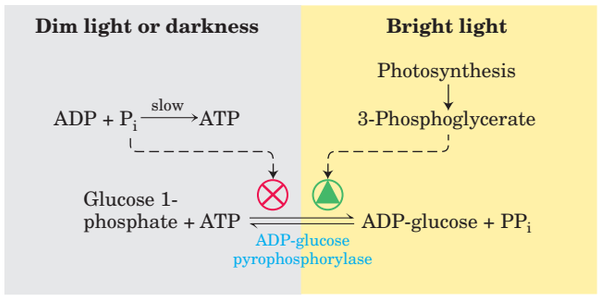

FIGURE 20–28 Regulation of ADP-glucose phosphorylase by 3-phosphoglycerate and P_i. This enzyme, which produces the precursor for starch synthesis, is rate-limiting in starch production. The enzyme is stimulated allosterically by 3-phosphoglycerate (3-PGA) and inhibited by P_i; in effect, the ratio [3-PGA]/[P_i], which rises with increasing rates of photosynthesis, controls starch synthesis at this step.

The key regulatory enzyme in starch synthesis is **ADP-glucose pyrophosphorylase** (Fig. 20–28); it is activated by 3-phosphoglycerate (which accumulates during active photosynthesis) and inhibited by P_i (which accumulates when light-driven condensation of ADP and P_i slows). When sucrose synthesis slows, 3-phosphoglycerate formed by CO_2 fixation accumulates, activating this enzyme and stimulating the synthesis of starch.

SUMMARY 20.3 Biosynthesis of Starch and Sucrose

- Starch synthase in chloroplasts and amyloplasts catalyzes the addition of single glucose residues, donated by ADP-glucose, to the reducing end of a starch molecule by a two-step insertion mechanism. Branches in amylopectin are introduced by a second enzyme.
- Sucrose is synthesized in the cytosol in two steps from UDP-glucose and fructose 1-phosphate.
- The partitioning of triose phosphates between sucrose synthesis and starch synthesis is regulated by fructose 2,6-bisphosphate (F2,6BP), an allosteric effector of the enzymes that determine the level of fructose 6-phosphate. F2,6BP concentration varies inversely with the rate of photosynthesis, and F2,6BP inhibits the synthesis of fructose 6-phosphate, the precursor to sucrose.

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20.4 Synthesis of Cell Wall Polysaccharides: Plant Cellulose and Bacterial Peptidoglycan

Cellulose is a major constituent of plant cell walls, providing strength and rigidity and preventing the swelling of the cell and rupture of the plasma membrane that might result when osmotic conditions favor water entry into the cell. Each year, worldwide, plants synthesize more than 10^{11} metric tons of cellulose, making this simple polymer one of the most abundant compounds in the biosphere. The structure of cellulose is simple: linear polymers of thousands of $\beta(1\rightarrow4)$-linked D-glucose units, assembled into bundles of about 36 chains, which aggregate side by side to form a microfibril (Fig. 20–29).

The biosynthesis of cellulose is less well understood than that of glycogen or starch. As a major component of the plant cell wall, cellulose must be synthesized from intracellular precursors but deposited and assembled outside the plasma membrane. The enzymatic machinery for initiation, elongation, and export of cellulose chains is more complicated than that needed to synthesize starch or glycogen (which are not exported). Bacteria face a similar set of problems when they synthesize the complex polysaccharides that make up their cell walls, and they may employ some of the same mechanisms to solve these problems.

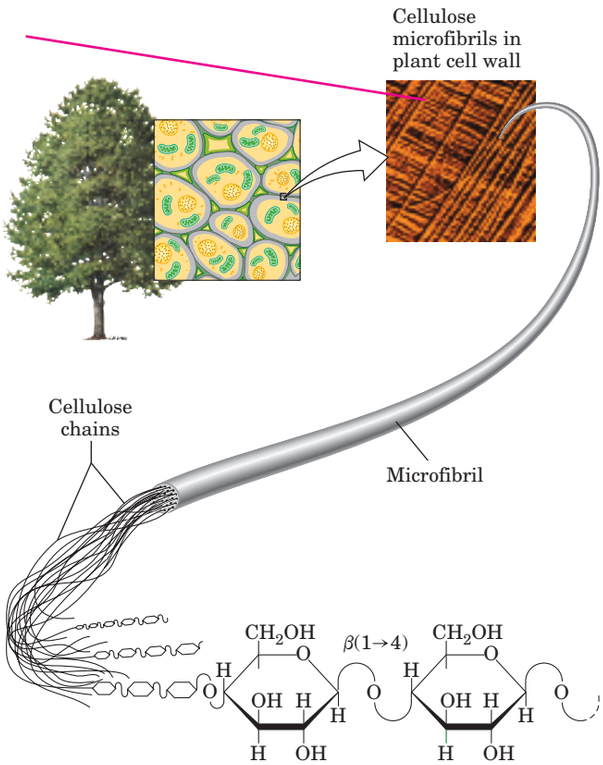

FIGURE 20–29 Cellulose structure. The plant cell wall is made up in part of cellulose molecules arranged side by side to form paracrystalline arrays—cellulose microfibrils. Many microfibrils combine to form a cellulose fiber, seen in the scanning electron microscope as a structure 5 to 12 nm in diameter, laid down on the cell surface in several layers distinguishable by the different orientations of their fibers.

Cellulose Is Synthesized by Supramolecular Structures in the Plasma Membrane

The complex enzymatic machinery that assembles cellulose chains spans the plasma membrane, with one part positioned to bind the substrate, UDP-glucose, in the cytosol and another part extending to the outside, responsible for elongating and crystallizing cellulose molecules in the extracellular space. Freeze-fracture electron microscopy shows these **terminal complexes**, also called **rosettes,** to be composed of six large particles arranged in a regular hexagon (Fig. 20–30). Several proteins, including the catalytic subunit of **cellulose synthase,** make up the terminal complex. Cellulose synthase has not been isolated in its active form, but its amino acid sequence has been determined from the nucleotide sequence of the gene that encodes it. From the primary structure we can use hydropathy plots (see Fig. 11–11) to deduce that the enzyme has eight transmembrane segments, connected by short loops on the outside, and several longer loops exposed to the cytosol. Much of the recent progress in understanding cellulose synthesis stems from genetic and molecular genetic studies of the plant *Arabidopsis thaliana*, which is especially amenable to genetic dissection and whose genome has been sequenced.

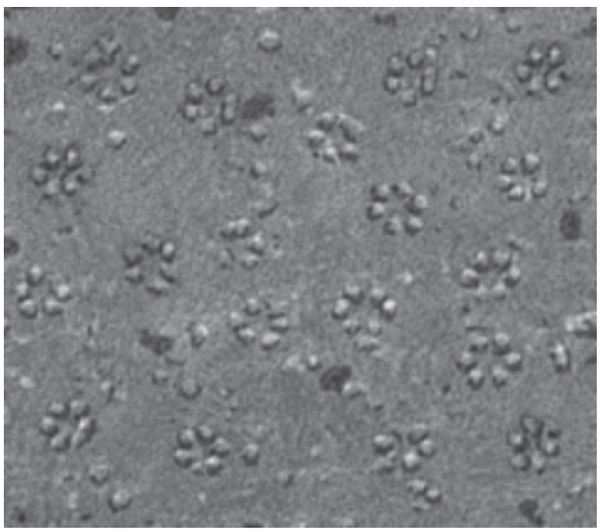

FIGURE 20–30 Rosettes. The outside surface of the plant plasma membrane in a freeze-fractured sample, viewed here with electron microscopy, contains many hexagonal arrays of particles about 10 nm in diameter, believed to be composed of cellulose synthase molecules and associated enzymes.

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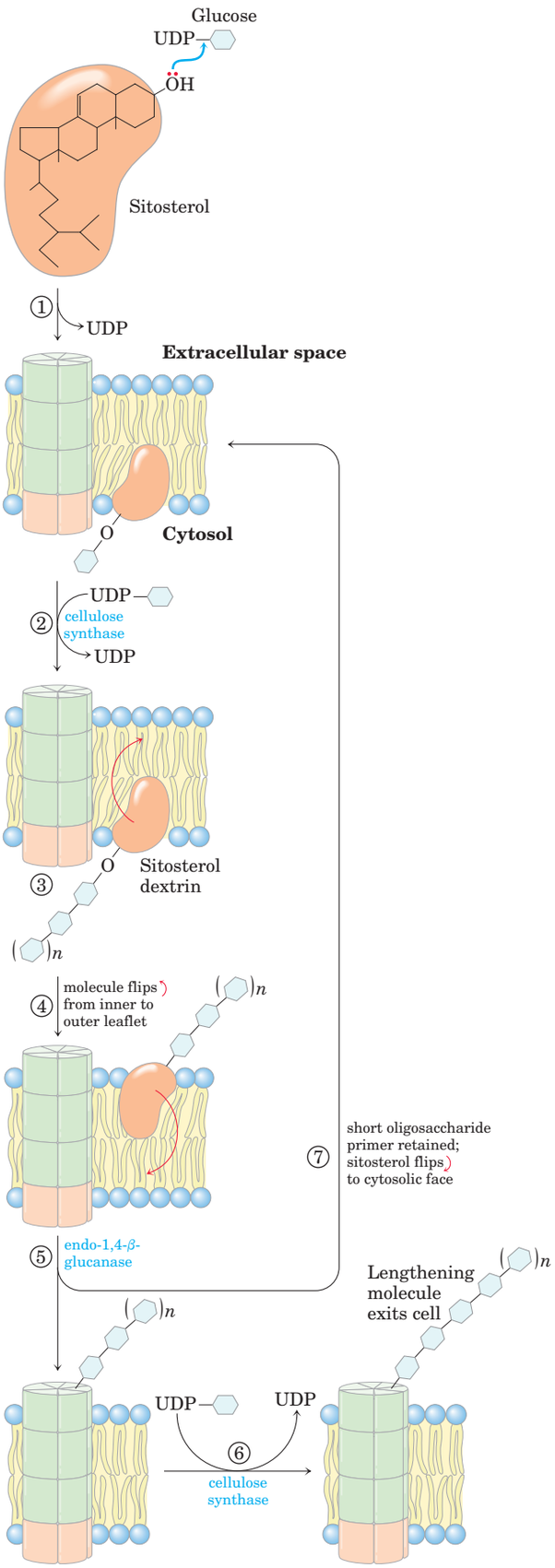

New cellulose chains appear to be initiated by the formation of a lipid-linked intermediate unlike anything involved in starch or glycogen synthesis. Glucose is transferred from UDP-glucose to a membrane lipid, probably the plant sterol sitosterol (Fig. 20–31), on the inner face of the plasma membrane. Here, intracellular cellulose synthase adds several more glucose residues to the first one, in (β1→4) linkage, forming a short oligosaccharide chain attached to the sitosterol (sitosterol dextrin). Next, the whole sitosterol dextrin flips across to the outer face of the plasma membrane, where most of the polysaccharide chain is removed by endo-1,4-β-glucanase. The shortened sitosterol dextrin primer now associates, perhaps covalently, with another form of cellulose synthase. Presumably this entire process occurs in the rosettes. Whether each of the 36 cellulose chains is initiated on its own lipid primer, or the primer recycles to start a number of chains, is not yet clear. In either case, the second form of cellulose synthase extends the polymer to 500 to 15,000 glucose units, extruding it onto the outer surface of the cell. The action of the enzyme is processive: one enzyme molecule adds many glucose units before releasing the growing cellulose chain. The direction of chain growth (whether addition occurs at the reducing end or at the nonreducing end) has not been established.

The finished cellulose is in the form of crystalline microfibrils (Fig. 20–29), each consisting of 36 separate cellulose chains lying side by side, all with the same (parallel) orientation of nonreducing and reducing ends. It seems likely that each particle in the rosette synthesizes six separate cellulose chains simultaneously and in parallel with the chains made by the other five particles, so that 36 polymers arrive together on the outer surface of the cell, already aligned and ready to crystallize as a microfibril of the cell wall. When the 36 polymers reach some critical length, their synthesis is terminated by an unknown mechanism; crystallization into a microfibril follows.

In addition to its catalytic subunit, cellulose synthase may have subunits that mediate extrusion of the polysaccharide chain (the pore subunit) and crystallization of the polysaccharide chains outside the cell

FIGURE 20–31 Lipid primer for cellulose synthesis. This proposed pathway begins with (1) the transfer of a glucosyl residue from UDP-glucose to a lipid "primer" (probably sitosterol) in the inner leaflet of the plasma membrane. After this initiation, (2) the chain of carbohydrate is elongated by transfer of glucosyl residues from UDP-glucose, until (3) a critical length of oligosaccharide is reached. (4) The sitosterol with its attached oligosaccharide now flips from the inner leaflet to the outer leaflet. (5) An endo-1,4-β-glucanase separates the growing chain from a short oligonucleotide still attached to the lipid. As it is pushed out of the cell, (6) the lipid-free polymer of glucosyl residues (the glucan acceptor) is further extended by the addition of glucosyl residues from UDP-glucose, catalyzed by cellulose synthase. (7) The lipid-linked oligosaccharide returns to serve as the primer for another chain of cellulose.

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(the crystallization subunit). The potent herbicide CGA 325′615, which specifically inhibits cellulose synthesis, causes rosettes to fall apart; the small amount of cellulose still synthesized remains tightly, perhaps covalently, bound to the catalytic subunit of cellulose synthase. The inhibitor may act by dissociating the catalytic subunit from the pore and crystallization subunits, preventing the later stages of cellulose synthesis.

The UDP-glucose used for cellulose synthesis is generated from sucrose produced during photosynthesis, by the reaction catalyzed by sucrose synthase (named for the reverse reaction):

$$\text{Sucrose} + \text{UDP} \longrightarrow \text{UDP-glucose} + \text{fructose}$$

In one proposed model, cellulose synthase spans the plasma membrane and uses cytosolic UDP-glucose as the precursor for extracellular cellulose synthesis. In another, a membrane-bound form of sucrose synthase forms a complex with cellulose synthase, feeding UDP-glucose from sucrose directly into cell wall synthesis (Fig. 20–32).

In the activated precursor of cellulose (UDP-glucose), the glucose is α-linked to the nucleotide, but in the product (cellulose), glucose residues are $\beta(1\rightarrow4)$-linked, so there is an inversion of configuration at the anomeric carbon (C-1) as the glycosidic bond forms. Glycosyltransferases that invert configuration are generally assumed to use a single-displacement mechanism, with nucleophilic attack by the acceptor species at the anomeric carbon of the donor sugar (UDP-glucose).

Certain bacteria (*Acetobacter*, *Agrobacteria*, *Rhizobia*, and *Sarcina*) and many simple eukaryotes also carry out cellulose synthesis, apparently by a mechanism similar to that in plants. If the bacteria use a membrane lipid to initiate new chains, it cannot be a sterol—bacteria do not contain sterols.

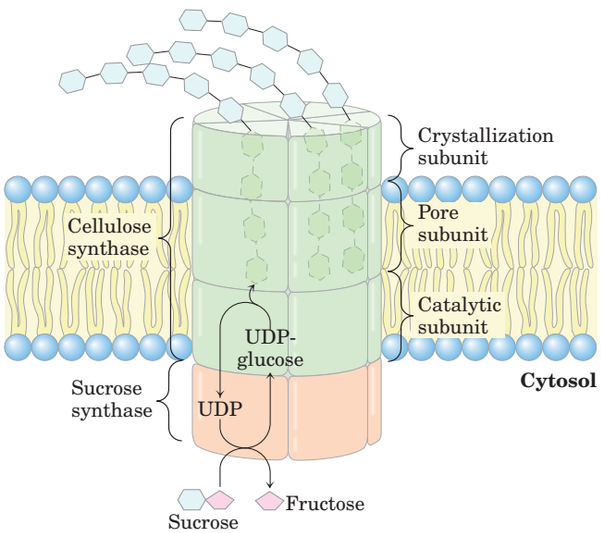

FIGURE 20–32 A plausible model for the structure of cellulose synthase. The enzyme complex includes a catalytic subunit with eight transmembrane segments and several other subunits that are presumed to act in threading cellulose chains through the catalytic site and out of the cell, and in the crystallization of 36 cellulose strands into the paracrystalline microfibrils shown in Figure 20–29.

Lipid-Linked Oligosaccharides Are Precursors for Bacterial Cell Wall Synthesis

Like plants, many bacteria have thick, rigid extracellular walls that protect them from osmotic lysis. The **peptidoglycan** that gives bacterial envelopes their strength and rigidity is an alternating linear copolymer of *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (Mur2Ac), linked by (β1→4) glycosidic bonds and cross-linked by short peptides attached to the Mur2Ac (Fig. 20–33). During assembly of the polysaccharide backbone of this complex macromolecule, both GlcNAc and Mur2Ac are activated by attachment of a uridine

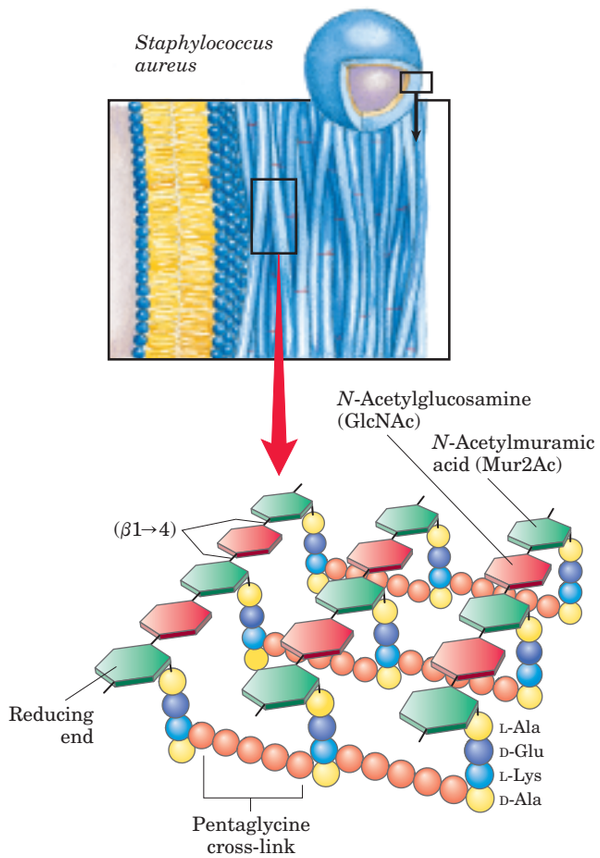

FIGURE 20–33 Peptidoglycan structure. This is the peptidoglycan of the cell wall of *Staphylococcus aureus*, a gram-positive bacterium. Peptides (strings of colored spheres) covalently link *N*-acetylmuramic acid residues in neighboring polysaccharide chains. Note the mixture of L and D amino acids in the peptides. Gram-positive bacteria such as *S. aureus* have a pentaglycine chain in the cross-link. Gram-negative bacteria, such as *E. coli*, lack the pentaglycine; instead, the terminal D-Ala residue of one tetrapeptide is attached directly to a neighboring tetrapeptide through either L-Lys or a lysine-like amino acid, diaminopimelic acid.

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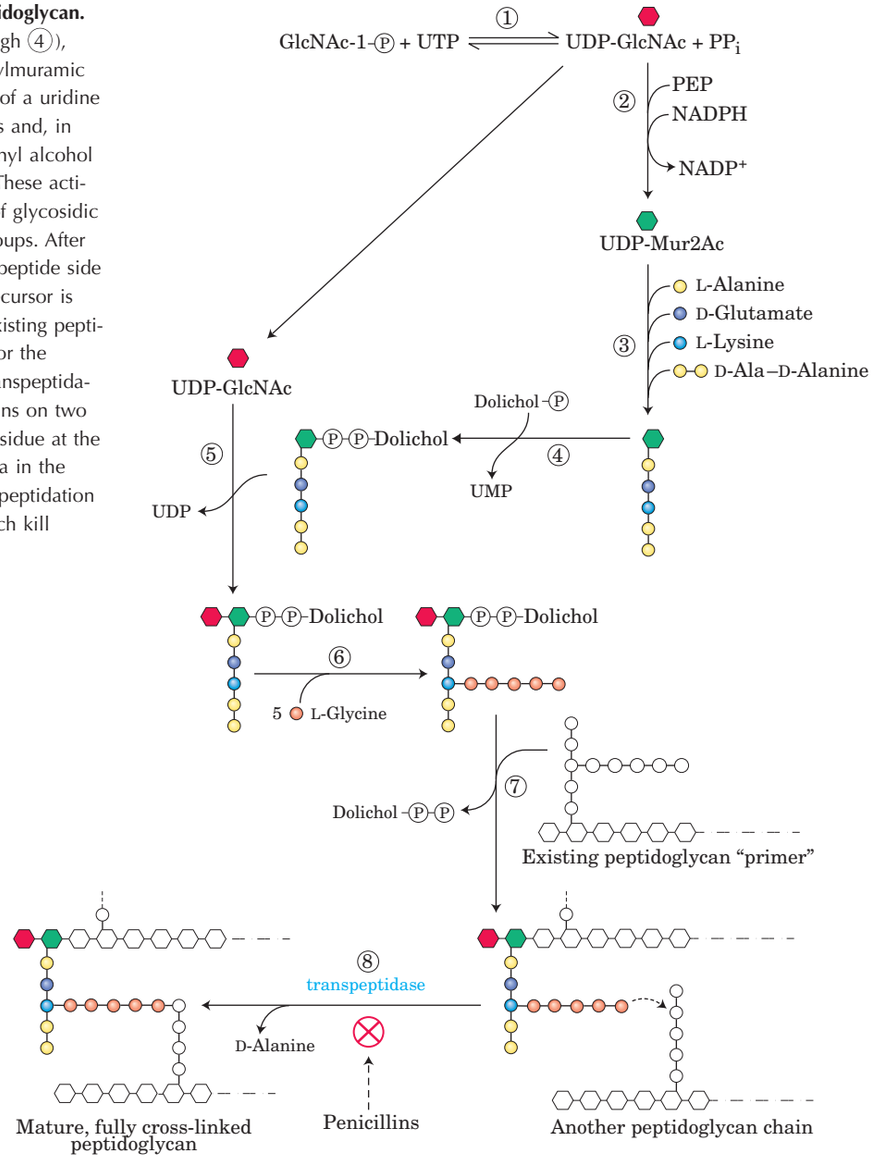

FIGURE 20–34 Synthesis of bacterial peptidoglycan. In the early steps of this pathway (① through ④), *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (Mur2Ac) are activated by attachment of a uridine nucleotide (UDP) to their anomeric carbons and, in the case of Mur2Ac, of a long-chain isoprenyl alcohol (dolichol) through a phosphodiester bond. These activating groups participate in the formation of glycosidic linkages; they serve as excellent leaving groups. After ⑤, ⑥ assembly of a disaccharide with a peptide side chain (10 amino acid residues), ⑦ this precursor is transferred to the nonreducing end of an existing peptidoglycan chain, which serves as a primer for the polymerization reaction. Finally, ⑧ in a transpeptidation reaction between the peptide side chains on two different peptidoglycan molecules, a Gly residue at the end of one chain displaces a terminal D-Ala in the other chain, forming a cross-link. This transpeptidation reaction is inhibited by the penicillins, which kill bacteria by weakening their cell walls.

nucleotide at their anomeric carbons. First, GlcNAc 1-phosphate condenses with UTP to form UDP-GlcNAc (Fig. 20–34, step ①), which reacts with phosphoenolpyruvate to form UDP-Mur2Ac (step ②); five amino acids are then added (step ③). The Mur2Ac-pentapeptide moiety is transferred from the uridine nucleotide to the membrane lipid dolichol, a long-chain isoprenoid alcohol (see Fig. 10–22f) (step ④), and a GlcNAc residue is donated by UDP-GlcNAc (step ⑤). In many bacteria, five glycines are added in peptide linkage to the amino group of the Lys residue of the pentapeptide (step ⑥). Finally, this disaccharide decapeptide is added to the nonreducing end of an existing peptidoglycan molecule (step ⑦). A transpeptidation reaction cross-links adjacent polysaccharide chains (step ⑧), contributing to a huge, strong, macromolecular wall around the bacterial cell. Many of the most effective antibiotics in use today act by inhibiting reactions in the synthesis of the peptidoglycan (Box 20–1).

Many other oligosaccharides and polysaccharides are synthesized by similar routes in which sugars are activated for subsequent reactions by attachment to nucleotides. In the glycosylation of proteins, for example (see Fig. 27–34), the precursors of the carbohydrate moieties include sugar nucleotides and lipid-linked oligosaccharides.

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BOX 20–1 BIOCHEMISTRY IN MEDICINE

The Magic Bullet versus the Bulletproof Vest: Penicillin and β-Lactamase

Because peptidoglycans are unique to bacterial cell walls, with no known homologous structures in mammals, the enzymes responsible for their synthesis are ideal targets for antibiotic action. Antibiotics that hit specific bacterial targets are sometimes called "magic bullets." Penicillin and its many synthetic analogs have been used to treat bacterial infections since these drugs came into wide application in World War II.

Penicillins and related antibiotics contain the β-lactam ring (Fig. 1), variously modified. All penicillins have a thiazolidine ring attached to the β-lactam, but they differ in the substituent at position 6, which accounts for the different pharmacological properties of the penicillins. For example, penicillin V is acid stable and can be administered orally, but methicillin is acid labile and must be given intravenously or intramuscularly. However, methicillin resists breakdown by bacterial enzymes (β-lactamases) whereas many other penicillins do not. The β-lactams have many of the properties that make a good drug. First, they target a metabolic pathway present in bacteria but not in people. Second, they have half-lives in the body long enough to be clinically useful. Third, they reach therapeutic concentrations in most, if not all, tissues and organs. Finally, they are effective against a broad range of bacterial species.

Penicillins block formation of the peptide crosslinks in peptidoglycans, acting as mechanism-based (suicide) inhibitors. The normal catalytic mechanism of the target enzyme activates the inhibitor, which then covalently modifies a critical residue in the active site. Transpeptidases employ a reaction mechanism (involving Ser residues) similar to that of chymotrypsin (see Fig. 6–21); the reaction activates β-lactams such as penicillin, which in turn inactivate the transpeptidases. After penicillin enters the transpeptidase active site, the proton on the hydroxyl group of an active-site Ser residue is abstracted to the nitrogen of the β-lactam ring, and the activated oxygen of the Ser hydroxyl attacks the carbonyl carbon at position 7 of the β-lactam, opening the ring and forming a stable penicilloyl-enzyme derivative that inactivates the enzyme (Fig. 2a).

Widespread use of antibiotics has driven the selection and evolution of antibiotic resistance in many pathogenic bacteria. The most important mechanism of resistance is inactivation of the antibiotic by enzymatic hydrolysis of the lactam ring, catalyzed by bacterial

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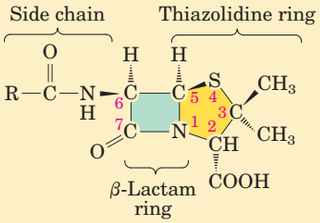

General structure of penicillins

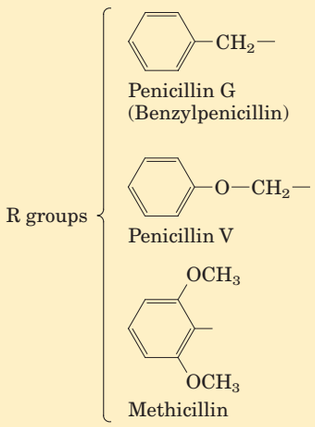

FIGURE 1

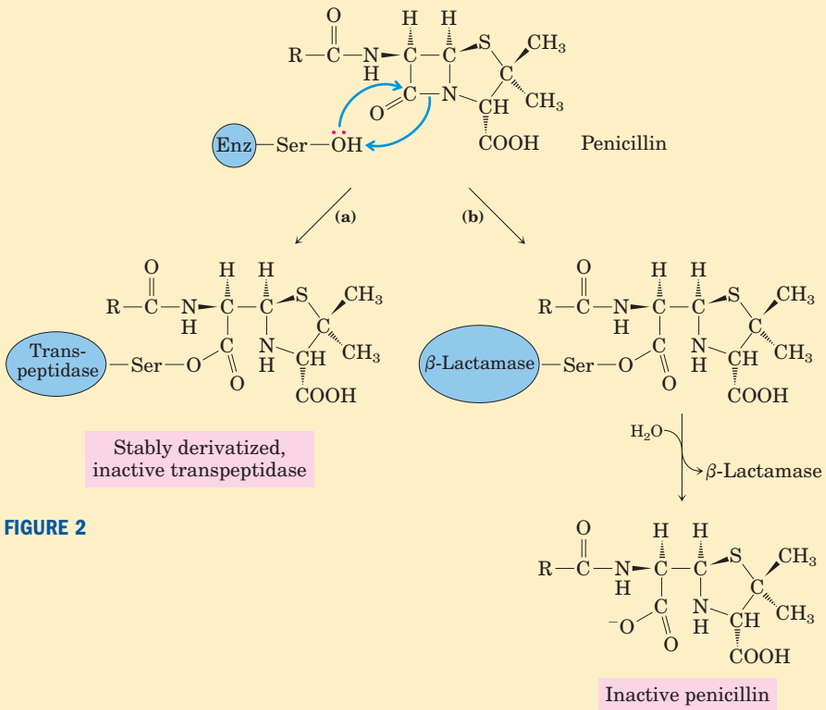

FIGURE 2

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BOX 20–1 BIOCHEMISTRY IN MEDICINE *(continued from previous page)*

β-lactamases, which provide bacteria with a bullet-proof vest (Fig. 2b). A β-lactamase forms a temporary covalent adduct with the carboxyl group of the opened β-lactam ring, which is immediately hydrolyzed, regenerating active enzyme. One approach to circumventing antibiotic resistance of this type is to synthesize penicillin analogs, such as methicillin, that are poor substrates for β-lactamases. Another approach is to administer along with antibiotics a β-lactamase inhibitor such as clavulanate or sulbactam.

Antibiotic resistance is a significant threat to public health. Some bacterial infections are now essentially untreatable with antibiotics. By the early 1990s, 20% to 40% of *Staphylococcus aureus* (the causative agent of "staph" infections) was resistant to methicillin, and 32% of *Neisseria gonorrhoeae* (the causative agent of gonorrhea) was resistant to penicillin. By 1986, 32% of *Shigella* (a pathogen responsible for severe forms of dysentery, some with a lethality of up to 15%) was resistant to ampicillin. Significantly, many of these pathogens are also resistant to many other antibiotics. In the future, we will need to develop new drugs that circumvent the bacterial resistance mechanisms or that act on different bacterial targets.

SUMMARY 20.4 Synthesis of Cell Wall Polysaccharides: Plant Cellulose and Bacterial Peptidoglycan

- Cellulose synthesis takes place in terminal complexes (rosettes) in the plasma membrane. Each cellulose chain begins as a sitosterol dextrin formed inside the cell. It then flips to the outside, where the oligosaccharide portion is transferred to cellulose synthase in the rosette and is then extended. Each rosette produces 36 separate cellulose chains simultaneously and in parallel. The chains crystallize into one of the microfibrils that form the cell wall.
- Synthesis of the bacterial cell wall peptidoglycan also involves lipid-linked oligosaccharides formed inside the cell and flipped to the outside for assembly.

20.5 Integration of Carbohydrate Metabolism in the Plant Cell

Carbohydrate metabolism in a typical plant cell is more complex in several ways than that in a typical animal cell. The plant cell carries out the same processes that generate energy in animal cells (glycolysis, citric acid cycle, and oxidative phosphorylation); it can generate hexoses from three- or four-carbon compounds by gluconeogenesis; it can oxidize hexose phosphates to pentose phosphates with the generation of NADPH (the oxidative pentose phosphate pathway); and it can produce a polymer of (α1→4)-linked glucose (starch) and degrade it to generate hexoses. But besides these carbohydrate transformations that it shares with animal cells, the photosynthetic plant cell can fix CO_2 into organic compounds (the rubisco reaction); use the products of fixation to generate trioses, hexoses, and pentoses (the Calvin cycle); and convert acetyl-CoA generated from fatty acid breakdown to four-carbon compounds (the glyoxylate cycle) and the four-carbon compounds to hexoses (gluconeogenesis). These processes, unique to the plant cell, are segregated in several compartments not found in animal cells: the glyoxylate cycle in glyoxysomes, the Calvin cycle in chloroplasts, starch synthesis in amyloplasts, and organic acid storage in vacuoles. The integration of events among these various compartments requires specific transporters in the membranes of each organelle, to move products from one organelle to another or into the cytosol.

Gluconeogenesis Converts Fats and Proteins to Glucose in Germinating Seeds

Many plants store lipids and proteins in their seeds, to be used as sources of energy and as biosynthetic precursors during germination, before photosynthetic mechanisms have developed. Active gluconeogenesis in germinating seeds provides glucose for the synthesis of sucrose, polysaccharides, and many metabolites derived from hexoses. In plant seedlings, sucrose provides much of the chemical energy needed for initial growth.

We noted earlier (Chapter 14) that animal cells can carry out gluconeogenesis from three- and four-carbon precursors, but not from the two acetyl carbons

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of acetyl-CoA. Because the pyruvate dehydrogenase reaction is effectively irreversible (pp. 602–603), animal cells have no way to convert acetyl-CoA to pyruvate or oxaloacetate. Unlike animals, plants and some microorganisms *can* convert acetyl-CoA derived from fatty acid oxidation to glucose. Some of the enzymes essential to this conversion are sequestered in glyoxysomes, where glyoxysome-specific isozymes of β-oxidation break down fatty acids to acetyl-CoA (see Fig. 16–22). The physical separation of the glyoxylate cycle and β-oxidation enzymes from the mitochondrial citric acid cycle enzymes prevents further oxidation of acetyl-CoA to CO_2. Instead, the acetyl-CoA is converted to succinate in the glyoxylate cycle (see Fig. 16–20). The succinate passes into the mitochondrial matrix, where it is converted by citric acid cycle enzymes to oxaloacetate, which moves into the cytosol. Cytosolic oxaloacetate is converted by gluconeogenesis to fructose 6-phosphate, the precursor of sucrose. Thus the integration of reaction sequences in three subcellular compartments is required for the production of fructose 6-phosphate or sucrose from stored lipids. Because only three of the four carbons in each molecule of oxaloacetate are converted to hexose in the cytosol, about 75% of the carbon in the fatty acids stored as seed lipids is converted to carbohydrate by the combined pathways of Figure 20–35. The other 25% is lost as CO_2 in the conversion of oxaloacetate to phosphoenolpyruvate. Hydrolysis of storage triacylglycerols also produces glycerol 3-phosphate, which can enter the gluconeogenic pathway after its oxidation to dihydroxyacetone phosphate (Fig. 20–36).

Glucogenic amino acids (see Table 14–4) derived from the breakdown of stored seed proteins also yield precursors for gluconeogenesis, following transamination and oxidation to succinyl-CoA, pyruvate, oxaloacetate, fumarate, and α-ketoglutarate (Chapter 18)—all good starting materials for gluconeogenesis.

Pools of Common Intermediates Link Pathways in Different Organelles

Although we have described metabolic transformations in plant cells in terms of individual pathways, these pathways interconnect so completely that we should instead consider pools of metabolic intermediates shared among these pathways and connected by readily reversible reactions (Fig. 20–37). One such **metabolite pool** includes the hexose phosphates glucose 1-phosphate, glucose 6-phosphate, and fructose 6-phosphate; a second includes the 5-phosphates of the pentoses ribose, ribulose, and xylulose; a third includes the triose phosphates dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Metabolite fluxes through these

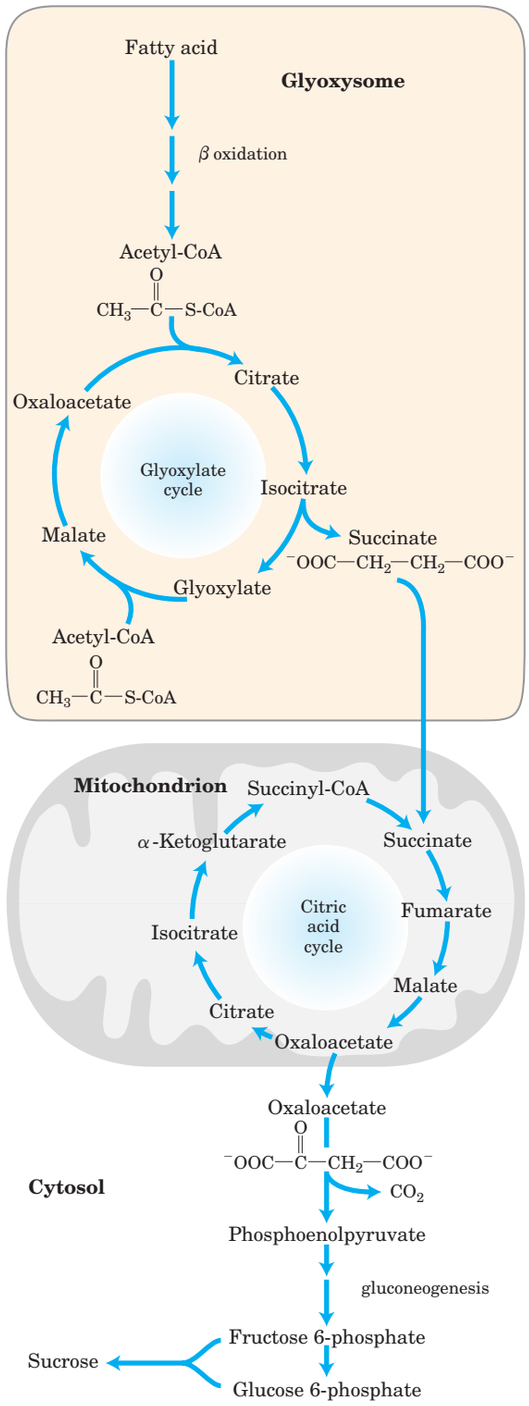

FIGURE 20–35 Conversion of stored fatty acids to sucrose in germinating seeds. This pathway begins in glyoxysomes. Succinate is produced and exported to mitochondria, where it is converted to oxaloacetate by enzymes of the citric acid cycle. Oxaloacetate enters the cytosol and serves as the starting material for gluconeogenesis and for the synthesis of sucrose, the transport form of carbon in plants.

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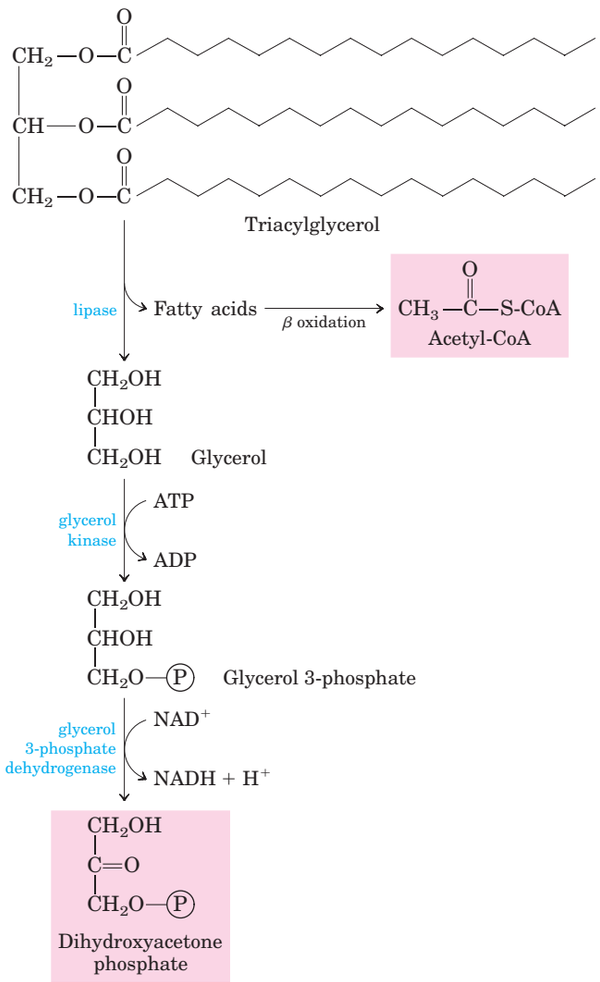

FIGURE 20–36 Conversion of the glycerol moiety of triacylglycerols to sucrose in germinating seeds. The glycerol of triacylglycerols is oxidized to dihydroxyacetone phosphate, which enters the gluconeogenic pathway at the triose phosphate isomerase reaction.

pools change in magnitude and direction in response to changes in the circumstances of the plant, and they vary with tissue type. Transporters in the membranes of each organelle move specific compounds in and out, and the regulation of these transporters presumably influences the degree to which the pools mix.

During daylight hours, triose phosphates produced in leaf tissue by the Calvin cycle move out of the chloroplast and into the cytosolic hexose phosphate pool, where they are converted to sucrose for transport to nonphotosynthetic tissues. In these tissues, sucrose is converted to starch for storage or is used as an energy source via glycolysis. In growing plants, hexose phosphates are also withdrawn from the pool for the synthesis of cell walls. At night, starch is metabolized by glycolysis to provide energy, essentially as in nonphotosynthetic organisms, and NADPH and ribose 5-phosphate are obtained through the oxidative pentose phosphate pathway.

SUMMARY 20.5 Integration of Carbohydrate Metabolism in the Plant Cell

- Plants can synthesize sugars from acetyl-CoA, the product of fatty acid breakdown, by the combined actions of the glyoxylate cycle and gluconeogenesis.
- The individual pathways of carbohydrate metabolism in plants overlap extensively; they share pools of common intermediates, including hexose phosphates, pentose phosphates, and triose phosphates. Transporters in the membranes of chloroplasts, mitochondria, amyloplasts, and peroxisomes mediate the movement of sugar phosphates between organelles. The direction of metabolite flow through the pools changes from day to night.

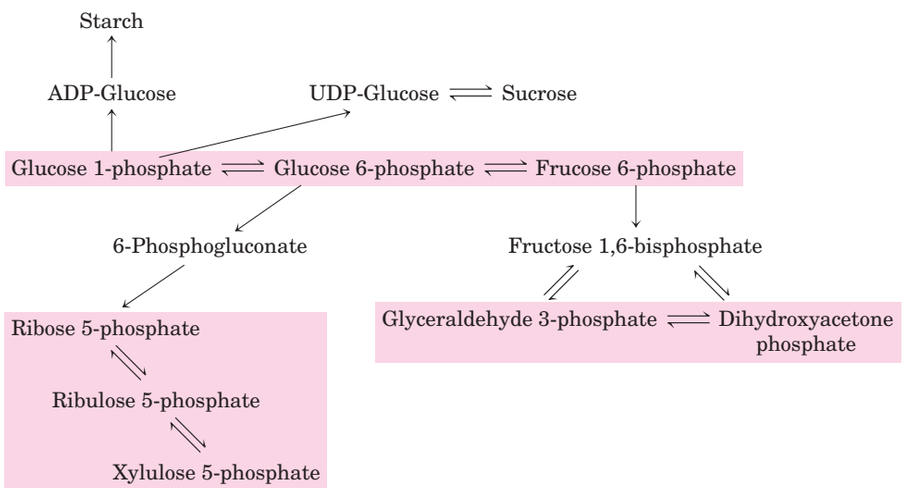

FIGURE 20–37 Pools of pentose phosphates, triose phosphates, and hexose phosphates. The compounds in each pool are readily interconvertible by reactions that have small standard free-energy changes. When one component of the pool is temporarily depleted, a new equilibrium is quickly established to replenish it. Movement of the sugar phosphates between intracellular compartments is limited; specific transporters must be present in an organelle membrane.

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Key Terms

Terms in bold are defined in the glossary.

Calvin cycle 752
plastids 752
chloroplast 752
amyloplast 752
carbon-fixation reaction 753
ribulose 1,5-bisphosphate 753
3-phosphoglycerate 753
pentose phosphate pathway 753
reductive pentose phosphate pathway 753
C_3 plants 754
ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco) 754
rubisco activase 757
transaldolase 759
transketolase 759
sedoheptulose 1,7-bisphosphate 760
ribulose 5-phosphate 760
carbon-assimilation reactions 763
thioredoxin 764
ferredoxin-thioredoxin reductase 764
photorespiration 766
2-phosphoglycolate 766
glycolate pathway 767
oxidative photosynthetic carbon cycle (C_2 cycle) 769
C_4 plants 769
phosphoenolpyruvate carboxylase 769
malic enzyme 769
pyruvate phosphate dikinase 769
CAM plants 770
nucleotide sugars 771
ADP-glucose 771
starch synthase 771
sucrose 6-phosphate synthase 772
fructose 2,6-bisphosphate 773
ADP-glucose pyrophosphorylase 774
cellulose synthase 775
peptidoglycan 777
metabolite pools 781

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Problems

1. Segregation of Metabolism in Organelles What are the advantages to the plant cell of having different organelles to carry out different reaction sequences that share intermediates?

2. Phases of Photosynthesis When a suspension of green algae is illuminated in the absence of CO_2 and then incubated with $^{14}CO_2$ in the dark, $^{14}CO_2$ is converted to [^{14}C]glucose for a brief time. What is the significance of this observation with regard to the CO_2-assimilation process, and how is it related to the light reactions of photosynthesis? Why does the conversion of $^{14}CO_2$ to [^{14}C]glucose stop after a brief time?

3. Identification of Key Intermediates in CO_2 Assimilation Calvin and his colleagues used the unicellular green alga *Chlorella* to study the carbon-assimilation reactions of photosynthesis. They incubated $^{14}CO_2$ with illuminated suspensions of algae and followed the time course of appearance

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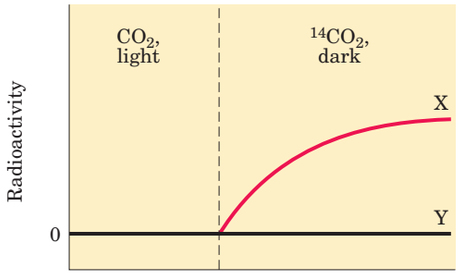
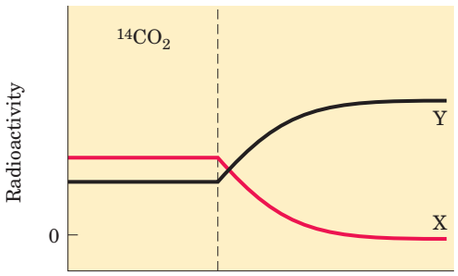
of ^{14}C in two compounds, X and Y, under two sets of conditions. Suggest the identities of X and Y, based on your understanding of the Calvin cycle.

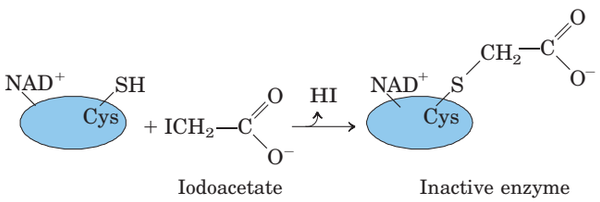
(a) Illuminated *Chlorella* were grown with unlabeled CO_2, then the light was turned off and $^{14}CO_2$ was added (vertical dashed line in the graph below). Under these conditions, X was the first compound to become labeled with ^{14}C; Y was unlabeled.



(b) Illuminated *Chlorella* cells were grown with $^{14}CO_2$. Illumination was continued until all the $^{14}CO_2$ had disappeared (vertical dashed line in the graph below). Under these conditions, X became labeled quickly but lost its radioactivity with time, whereas Y became more radioactive with time.



4. Regulation of the Calvin Cycle Iodoacetate reacts irreversibly with the free —SH groups of Cys residues in proteins.



Predict which Calvin cycle enzyme(s) would be inhibited by iodoacetate, and explain why.

5. Thioredoxin in Regulation of Calvin Cycle Enzymes Motohashi and colleagues* used thioredoxin as a hook to fish out from plant extracts the proteins that are activated by thioredoxin. To do this, they prepared a mutant thioredoxin in which one of the reactive Cys residues was replaced with a Ser. Explain why this modification was necessary for their experiments.

6. Comparison of the Reductive and Oxidative Pentose Phosphate Pathways The *reductive* pentose phosphate pathway generates a number of intermediates identical to those of the *oxidative* pentose phosphate pathway (Chapter 14). What role does each pathway play in cells where it is active?

7. Photorespiration and Mitochondrial Respiration Compare the oxidative photosynthetic carbon cycle (C_2 cycle), also called *photorespiration,* with the *mitochondrial respiration* that drives ATP synthesis. Why are both processes referred to as respiration? Where in the cell do they occur, and under what circumstances? What is the path of electron flow in each?

8. Rubisco and the Composition of the Atmosphere N. E. Tolbert† has argued that the dual specificity of rubisco for CO_2 and O_2 is not simply a leftover from evolution in a low-oxygen environment. He suggests that the relative activities of the carboxylase and oxygenase activities of rubisco actually have set, and now maintain, the ratio of CO_2 to O_2 in the earth's atmosphere. Discuss the pros and cons of this hypothesis, in molecular terms and in global terms. How does the existence of C_4 organisms bear on the hypothesis?

9. Role of Sedoheptulose 1,7-Bisphosphatase What effect on the cell and the organism might result from a defect in sedoheptulose 1,7-bisphosphatase in (a) a human hepatocyte and (b) the leaf cell of a green plant?

10. Pathway of CO_2 Assimilation in Maize If a maize (corn) plant is illuminated in the presence of $^{14}CO_2$, after about 1 second, more than 90% of all the radioactivity incorporated in the leaves is found at C-4 of malate, aspartate, and oxaloacetate. Only after 60 seconds does ^{14}C appear at C-1 of 3-phosphoglycerate. Explain.

11. Identifying CAM Plants Given some $^{14}CO_2$ and all the tools typically present in a biochemistry research lab, how would you design a simple experiment to determine whether a plant was a typical C_4 plant or a CAM plant?

12. Chemistry of Malic Enzyme: Variation on a Theme Malic enzyme, found in the bundle-sheath cells of C_4 plants, carries out a reaction that has a counterpart in the citric acid cycle. What is the analogous reaction? Explain your choice.

13. The Cost of Storing Glucose as Starch Write the sequence of steps and the net reaction required to calculate the cost, in ATP molecules, of converting a molecule of cytosolic glucose 6-phosphate to starch and back to glucose 6-phosphate. What fraction of the maximum number of ATP molecules available from complete catabolism of glucose 6-phosphate to CO_2 and H_2O does this cost represent?

*Motohashi, K., Kondoh, A., Stumpp, M.T., & Hisabori, T. (2001) Comprehensive survey of proteins targeted by chloroplast thioredoxin. *Proc. Natl. Acad. Sci. USA* **98,** 11,224–11,229.

†Tolbert, N.E. (1994) The role of photosynthesis and photorespiration in regulating atmospheric CO_2 and O_2. In *Regulation of Atmospheric CO_2 and O_2 by Photosynthetic Carbon Metabolism* (Tolbert, N.E. & Preiss, J., eds), pp. 8–33, Oxford University Press, New York.

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14. Inorganic Pyrophosphatase The enzyme inorganic pyrophosphatase contributes to making many biosynthetic reactions that generate inorganic pyrophosphate essentially irreversible in cells. By keeping the concentration of PP_i very low, the enzyme "pulls" these reactions in the direction of PP_i formation. The synthesis of ADP-glucose in chloroplasts is one reaction that is pulled in the forward direction by this mechanism. However, the synthesis of UDP-glucose in the plant cytosol, which produces PP_i, is readily reversible in vivo. How do you reconcile these two facts?

15. Regulation of Starch and Sucrose Synthesis Sucrose synthesis occurs in the cytosol and starch synthesis in the chloroplast stroma, yet the two processes are intricately balanced. What factors shift the reactions in favor of (a) starch synthesis and (b) sucrose synthesis?

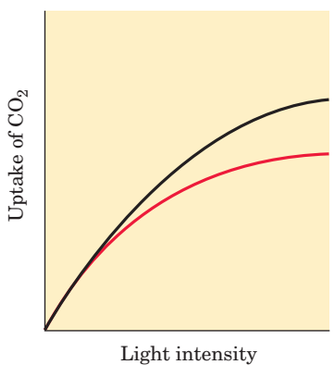
16. Regulation of Sucrose Synthesis In the regulation of sucrose synthesis from the triose phosphates produced during photosynthesis, 3-phosphoglycerate and P_i play critical roles (see Fig. 20–26). Explain why the concentrations of these two regulators reflect the rate of photosynthesis.

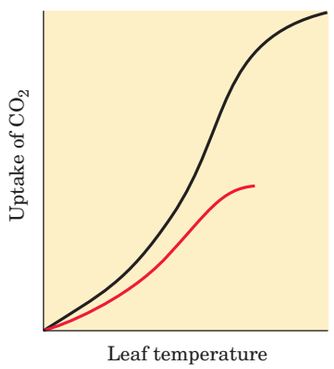
17. Sucrose and Dental Caries The most prevalent infection in humans worldwide is dental caries, which stems from the colonization and destruction of tooth enamel by a variety of acidifying microorganisms. These organisms synthesize and live within a water-insoluble network of dextrans, called dental plaque, composed of (α1→6)-linked polymers of glucose with many (α1→3) branch points. Polymerization of dextran requires dietary sucrose, and the reaction is catalyzed by a bacterial enzyme, dextran-sucrose glucosyltransferase.

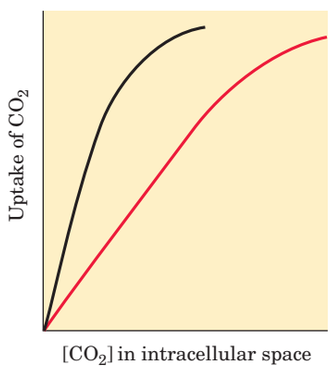
(a) Write the overall reaction for dextran polymerization.

(b) In addition to providing a substrate for the formation of dental plaque, how does dietary sucrose also provide oral bacteria with an abundant source of metabolic energy?

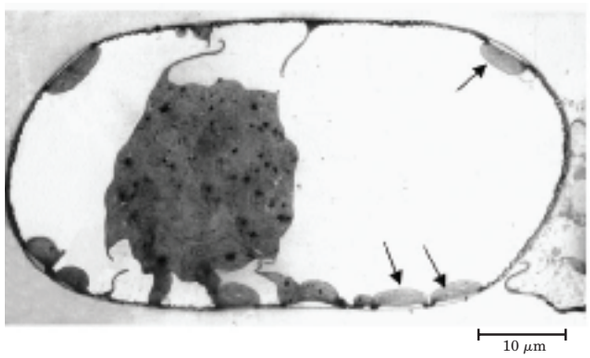
18. Differences between C_3 and C_4 Plants The plant genus *Atriplex* includes some C_3 and some C_4 species. From the data in the plots below (species 1, black curve; species 2, red curve), identify which is a C_3 plant and which is a C_4 plant. Justify your answer in molecular terms that account for the data in all three plots.







19. C_4 Pathway in a Single Cell In typical C_4 plants, the initial capture of CO_2 occurs in one cell type, and the Calvin cycle reactions occur in another (see Fig. 20–23). Voznesenskaya and colleagues[††] have described a plant, *Bienertia cycloptera*—which grows in salty depressions of semidesert in Central Asia—that shows the biochemical properties of a C_4 plant but unlike typical C_4 plants does not segregate the reactions of CO_2 fixation into two cell types. PEP carboxylase and rubisco are present in the same cell. However, the cells have two types of chloroplasts, which are localized differently, as shown in the micrograph. One type, relatively poor in grana (thylakoids), is confined to the periphery; the more typical chloroplasts are clustered in the center of the cell, separated from the peripheral chloroplasts by large vacuoles. Thin cytosolic bridges pass through the vacuoles, connecting the peripheral and central cytosol.



In this plant, where would you expect to find (a) PEP carboxylase, (b) rubisco, and (c) starch granules? Explain your answers with a model for CO_2 fixation in these C_4 cells.

[††]Voznesenskaya, E.V., Fraceschi, V.R., Kiirats, O., Artyusheva, E.G., Freitag, H., & Edwards, G.E. (2002) Proof of C_4 photosynthesis without Kranz anatomy in *Bienertia cycloptera* (Chenopodiaceae). *Plant J.* **31,** 649–662.